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PROGRESS IN THE STUDY OF THE ANCIENT SEDIMENTS.

IN the latter half of the year 1894, considerable additions were made to our knowledge of the ancient sediments, both at home and abroad, though no authenticated discovery of exceptional importance was announced. Somewhat sensational announcements appeared during that time, but the results of greatest value are due to steady work at points of detail, for these results are not given to the world until they have received ample confirmation at the hands of the workers. It is pleasing to find that British geologists have done their full share in carrying on work of this nature, so that year by year the "records of the rocks," both at home and abroad, become appreciably less imperfect.

Taking into account, in the first place, treatises which appeared during the second half of last year, which throw new light on more than one of the great divisions of the older rocks, we may notice at the outset the very important results obtained by various members of her Majesty's Geological Survey as recorded in the official report (1). It is there announced that the Torridonian rocks, previously proved by the surveyors to be of Precambrian age, are divisible into four groups, as follows in descending order:—

4. Cailleach Head Group, 1000-1500 feet sandstones, flags, shales, and calcareous bands.

3. Aultbea Group, 2000-3000 feet chocolate and red sandstones, flags, and shaley partings.

2. Applecross Group, 4000-5000 feet coarse arkose.

1. Diabeg Group, 5000 feet and upwards red sandstones, greywackes, shales, limestones, and calcareous bands. The Diabeg group is further divided by Clough into four subgroups.

In Ireland equivalents of the Precambrian and Lower Palæozoic rocks of Scotch type are gradually being mapped in detail. Much information is given concerning the Southern Highlands of Scotland, including the discovery by Barrow of radiolarian cherts in Forfarshire and Kincardineshire on the edge of the Highland border. Lamplugh has shown that in the Isle of Man the "Skiddaw slates" are everywhere folded, faulted, and sheared, and that the apparent great thickness of these rocks in the island is therefore deceptive, a conclusion which seems to bear out a similar statement previously made with regard to the "Skiddaw slates" of the Lake District. In the Isle of Man slates, coarse breccias are found, and these are considered by Lamplugh to be friction breccias. In Devonshire the Devonian rocks of the Plymouth area have been brought into connection with those of Newton Abbot and Torquay. One of the most interesting announcements in the report is that explorations in Cheshire and South Lancashire "appear to prove that in both counties the Lower Mottled Sandstone, hitherto taken as the base of the Bunter, belongs to the Permian series, for above it, and below the Bunter pebble beds, lie marls with *Schizodus*". No doubt all these points will receive fuller treatment in detailed memoirs, and indeed a paper has already been read, though not yet published, on the breccias of the Skiddaw slates of the Isle of Man.

Passing from Britain to her possessions, it is interesting to have the mature judgment of the veteran Sir William Dawson on various disputed points (2). Concerning the Precambrian rocks he remarks that "the older gneissic group of the West Highlands of Scotland does not contain the whole of the Laurentian of Logan, the Lewisian of Murchison, but only or mainly the lower part of it, the Ottawa group of the Canadian Survey. A certain limited

tract at Loch Maree not improbably represents the Upper Laurentian or Grenville series, and this certainly occurs in the western islands. . . . The Torridon sandstones and the associated beds . . . seem . . . to be equivalent to the Huronian of Logan. . . . The Uriconian and Longmyndian of Geikie probably include the equivalents of our Kewenian, and the same may perhaps be said of the Dalradian of Scotland. . . . Some portions of these rocks may, however, be the same with what in Canada has been called by Matthew 'basal Cambrian'." Without commenting further on the vexed question of the correlation of the Precambrian rocks of different areas, which will remain in a state of uncertainty until far more work has been done amongst the rocks, we may pass on to notice a sentence occurring in Sir William's criticism of the theories of coal-making: "The coal-deposits of the great Carboniferous system . . . mark a special stage in the production of our continents, when they were less differentiated as to orography, and when a very uniform and equable climate extended over the northern hemisphere accompanied by a very peculiar vegetation. Such conditions did not occur in combination and to a like extent in any succeeding period in the earth's history." This sentence contains a note of warning which some writers will do well to consider. Extreme uniformitarians sometimes appear to forget that in every system, differentiation has proceeded to a greater extent than in the preceding one, and that as with the Carboniferous so with the other systems, the conditions do not "occur in combination, and to a like extent in any succeeding period".

Having passed over the Atlantic we may pause there before returning to the Old World to notice two papers treating of kindred subjects, the occurrence of certain conglomerates amongst the Palæozoic rocks. The first of these treats of "Palæozoic Overlaps in Montgomery and Pulaski Counties, Virginia" (3).

The rocks described in this paper are in ascending order:—

1. *Cambrian.* The Graysonton Formation, a mass of

red and green shales and interbedded limestones generally siliceous, containing no fossils; their exact age is therefore as yet unknown.

2. *Cambro-Silurian.* Shenandoah Limestone, 4000 feet thick, generally a grey dolomite much folded and crushed, at the top are a few feet of blue sparingly fossiliferous limestone, probably the only representatives of the Trenton-Chazy limestones in this region. The limestones contain conglomerates with pebbles similar in character to the matrix. With reference to the nature of this conglomerate, the writer states that "its meaning is obscure".

3. *Silurian.* Sevier (Hudson River Shale) contains fossiliferous limestones. It is not quite clear why this formation is placed by the writer in the Silurian, if he uses the term "Cambro-Silurian" in the sense of Ordovician. It is succeeded by the Church (Medina-Oneida) sandstone, the fossiliferous Rockwood (Clinton) formation, and the Giles (Lower Helderberg and Oriskany) formation (the Oriskany and all save the lowest subdivision of the Lower Helderberg are usually included in the Devonian).

4. *Devonian.* Walker Black Shale and Kimberley Shale.

5. *Carboniferous.* Price (Pocono) Sandstone closely resembles the upper Coal Measures of the Appalachian basin and is succeeded by the Pulaski shale.

The author describes five periods of Appalachian folding, the first being in Lower Cambrian times, the second near the top of the Shenandoah limestone, the third which was the main period of deformation occurring between the Devonian and Silurian periods, the fourth near the middle of the Carboniferous, and the fifth, post-Carboniferous. The last has been generally considered the most important of the Appalachian foldings, though "it now seems as though this was no more important than many which preceded it, and that in fact the deformation has been practically continuous since early Palæozoic time".

The second paper to which allusion has been made is on "Palæozoic Intra-formational Conglomerates" (4). In this various palæozoic conglomerates are described, with pebbles

which often contain a fauna similar to that of the matrix in which the pebbles are included. These chiefly occur in the case of limestone. The author observes that "the history of Appalachian sedimentation and mountain-building proves that a more or less constant movement was taking place from Algonkian[1] time to the close of the Palæozoic. This movement was at times greatly prolonged and resulted in marked topographic features. More frequently the minor movements produced local effects, and some of them resulted in the formation of the conglomerates described."

These papers have been noticed at some length because they appear to confirm results obtained by geologists in the Old World that orogenetic movements occurred during much longer periods of time than was once supposed, though each great uplift was not necessarily the result of continuous movement. At the same time it is not clear that all the conglomerates described in these papers are true deposits. The writer of this article has had frequent occasion of late years to study friction-breccias and friction-conglomerates, and he finds that the latter are specially prone to occur in limestones. In such cases the occurrence of a similar fauna in the matrix and the pebbles would be readily explicable. The statement of one of the above authors that the meaning of one of the conglomerates "is obscure" encourages one to suggest the probability of the occurrence of friction-conglomerates amongst the highly disturbed rocks of the region described, for they are far from rare amongst the disturbed regions of Europe, and frequently simulate conglomerates of deposition with extraordinary fidelity.

A paper by M. Marcel Bertrand (5) on the Alps is chiefly devoted to the foldings and their effects, but contains information about the Alpine Carboniferous rocks. Another Alpine paper (6) treats of a district which has previously been described at considerable length by Lory; in this paper also the systems of folds are discussed in detail. A

[1] Late Precambrian.

description is given of the Precambrian rocks, consisting of hornblendic gneisses, amphibolites, and mica-schists (both granulitic and non-granulitic). Carboniferous rocks (grès à Anthracite) appear on each side of the principal ridge in the form of two regular synclines (an eastern and a western), which seem to correspond with two separate basins occupying depressions on each side of a precarboniferous chain. Some of these Carboniferous beds have suffered considerable metamorphism, and the author gives petrological descriptions of the macroscopic and microscopic characters of a number of new types of these metamorphic rocks, including highly altered conglomerates, sandstones, etc. In most cases sericite is the only new mineral produced by the metamorphism. Orthophyric volcanic rocks are described; stratigraphical observations lead the author to consider that most of the lavas occur at the top of the Carboniferous deposits of the Grandes-Rousses Massif. A description of the systems of folding is given, the Hercynian folds run generally north and south, as does one set of the Alpine folds, whilst another set of the latter runs east and west. The paper is a valuable contribution to the effects of dynamic action in producing metamorphism of recognisable sediments.

Taking now into account the papers treating of special deposits or groups of deposits, we do not find many dealing with important questions concerning Precambrian rocks. Bell (7) describes some remarkable outliers of Potsdam beds and Black River limestones in depressions of granite. He suggests that these depressions are due to submarine weathering. Much of the Archæan rock must have been denuded before the deposition of the Palæozoic rocks upon it, and the interval between them is considered to have been probably much greater than has generally been supposed. It is to be observed, however, that the presence of Black River beds immediately succeeding Precambrian rocks, indicates further weathering of these rocks in Palæozoic times before the deposition of the Black River limestone.

Wiman (8) describes as organic a problematical body

from the Visingö group which lies between the "Urgebirge" and the Lower Palæozoic rocks of Scandinavia. There is no doubt, as has been before remarked, that Precambrian fossils will be found to exist when these rocks have been more fully studied, but it is very necessary, considering the many occasions on which the existence of these fossils has been maintained on insufficient evidence, that very conclusive evidence should be brought forward before the actual discovery of Precambrian fossils is taken as generally established. At present the evidence for the existence of Precambrian fossils is very much in the same condition as that of the discovery of human bones in palæolithic deposits. It is generally admitted that man existed in palæolithic times from evidence other than that of the bones, some of the human bones that have been claimed as of palæolithic age may possibly, nay, probably, be of that age, whilst the greater number of them certainly are newer, and in the opinion of many competent authorities the actual proofs of the palæolithic age of any one human bone remain to be given. So with Precambrian fossils, the only difference being that in the case of the palæolithic period we have actual evidence of the existence of man furnished by implements, whereas in that of the Precambrian fossils, the existence of organisms in Precambrian times is inferential, the great complexity of the earliest Cambrian (*Olenellus*) fauna rendering it practically certain that earlier faunas existed on the earth. It will be remembered that in addition to the case described by Wiman, the existence of Precambrian fossils has been recently asserted in the phyllades of St. Lô and the Torridonian of the Scottish Highlands. The fossils stated to occur in the latter beds have not yet been described, and, as noted in a previous article, doubt has been thrown on the organic nature of those from the former. Perhaps the least doubtful of the many recorded instances of Precambrian fossils is that about to be noticed. I refer to a paper by Noetling (9). He describes the following beds in descending order :—

4. Bhanganwalla group, or salt-crystal pseudomorph zone.

3. Jutana group, or Magnesian sandstone.
2. Khussak group, or *Neobolus* beds.
1. Khewsa group, or purple sandstone.

The whole forming the Punjab "province" of Cambrian beds. The purple sandstone is stated to be not clearly separable from the *Neobolus* beds. The latter consist of dolomitic sandstone alternating with shales, and have a thickness of from twenty to one hundred and fifty feet. They are split up into five subdivisions in descending order :—

E. Zone of *Olenellus* sp., containing also *Trimerellidæ*.
D. Zone of *Neobolus Warthi*.
C. Upper Annelid sandstone.
B. Zone of *Hyolithes Wynnei*.
A. Lower Annelid sandstone.

He maintains that he confirms Waagen's view that the fauna of the *Neobolus* shales is explicable by the fact that the fauna is older than any known Cambrian fauna, occurring as it does below the *Olenellus* beds, in which case *Neobolus Warthi*, *N. Wynnei*, and *Hyolithes Wynnei* must be considered the earliest known forms of animal life. The Magnesian sandstone is separated into five subdivisions on lithological characters. It contains *Stenotheca* and is believed to be Lower Cambrian. No fossils have been found in the salt-crystal pseudomorph group.

A few observations may be offered on the facts contained in this paper. In the first place, the concurrence of two authorities of such repute as Waagen and Noetling in maintaining that the *Neobolus* beds contain a fauna older than any known fauna is of very great importance, nor is its importance lessened by the fact that these authorities do not altogether agree concerning minor points. At the same time, it must be pointed out that the evidence is of a negative character. *Olenellus* has been found in the uppermost subdivision of the *Neobolus* beds, but not in the lower ones, still it is quite possible that the genus and its associates may eventually be found therein, especially when we take into account the small thickness of the group, and the similarity of characters of the different divisions which is

sufficiently great to allow them to be included in one group. At least two horizons of *Olenellus* are known elsewhere as proved by Schmidt in the year 1888, and considering that the *Olenellus* group is comparable in importance with the *Paradoxides* group, which is divisible into several zones characterised by different species of *Paradoxides*, as established by the researches of Hicks, Brögger, and Tullberg, and also that so many species of *Olenellus* are now known, we may well suppose that the *Olenellus* beds like those containing the *Paradoxides* fauna may be separated into several zones characterised by different species of *Olenellus*. It has not yet been made clear that the *Neobolus* beds are not the equivalents of some of these, for the fossils associated with *Olenellus* have not yet been described from all the areas where *Olenellus* is found.

Should the *Neobolus* beds be definitely proved to be lower than the *Olenellus* beds, the question as to where the base of the Cambrian system should be drawn, which is bound to confront us sooner or later, becomes a matter of pressing importance, for it will not do to absorb group after group into the Cambrian rocks as they are found to be fossiliferous.

The Cambrian system as defined by Sedgwick is typically developed in North Wales, where its base is formed by the Harlech and Llanberis beds. In these North Welsh beds the *Paradoxides* fauna is known to occur, but the existence of strata of the age of the *Olenellus* beds is only inferred, no definite fossils of *Olenellus* age having yet been detected therein. Still, the *Olenellus* beds have so constantly been referred to the Cambrian system that it would introduce additional confusion to relegate them to an earlier system, should it be ultimately proved (which is unlikely) that none of the beds of Sedgwick's typical area appertain to the beds of *Olenellus* age. Also considering that the *Olenellus*, *Paradoxides*, and *Olenus* faunas are of approximately equal importance, the beds containing them make a convenient system as the term is understood amongst geologists. But the inclusion of beds of earlier age than the *Olenellus* beds (such beds being probably not represented in North Wales)

in the Cambrian system is to be deprecated, and the writer would suggest that any fossiliferous margins which are in future proved to be of an age antecedent to that of the *Olenellus* beds should be placed in a system earlier than the Cambrian.

A paper of interest to British geologists (10) adds to our knowledge of the ridge which existed in Central England in early Carboniferous times. Prof. Hull records the results of a boring which passed through 262 feet of Bunter Sandstone and 514 feet of Coal Measures, and reached a reddish-purple and grey grit and micaceous quartzite which was pierced to a depth of 19 feet from its summit. He considers that this probably belongs to the Lower Cambrian, a view with which Prof. Lapworth concurs, in which case the Lower Coal Measures and all the Carboniferous rocks beneath them, as well as the Devonian, Silurian, Ordovician and higher Cambrian rocks, are absent.

A valuable historical sketch, treating exclusively of the Cambrian rocks, appeared in the latter half of 1894 (11). In this paper Dr. Hicks gives an account of the progress of work during thirty years as concerned with the discovery of the various life-zones of the Cambrian rocks. Though the paper is a historical sketch, and not a record of new observations, it is of importance to the student not merely as an epitome of our present knowledge of the Cambrian strata, but also as an indication of how that knowledge was obtained, and is therefore particularly suggestive of lines along which research may be carried in the immediate future.

Several contributions to the palæontology of the Ordovician and Silurian rocks have been made in the period under notice. Počta (12) gives another instalment of the great work commenced and carried out for so many years by Barrande; he describes the Bryozoa of the Lower Palæozoic area of Bohemia. F. Schmidt (13) makes another contribution to his accurate descriptions of the trilobites of Esthonia and Livonia. In this memoir he treats of the genera *Calymene*, *Bronteus*, *Proetus*, *Cyphaspis*, *Menocephalus*, *Harpides*, *Harpes*, *Trinucleus*, *Ampyx*,

Remopleurides, and *Agnostus*. He adopts three groups for the genus *Calymene*, *viz.*, *Calymene* proper, *Pharostoma corda*, and *Ptychometopus* n. subgen., and gives diagnoses of them. T. S. Hall (14) has a "Note on the Distribution of the Graptolitidæ in the Rocks of Castlemaine (Australia)". Some of the identifiable forms, according to the author, range through the series, whilst others afford a ready means of distinguishing certain facies. As *Tetragraptus* ranges throughout the beds, they would seem to be of Arenig age notwithstanding the occurrence of "what is apparently a *Monograptus*". The forms recorded are similar to those of the Skiddaw slates of England and the Quebec group of Canada, and appear to be restricted to certain horizons of the strata. With regard to the apparent *Monograptus*, it may be suggested that it is possibly an *Azygograptus*—*Monograptus* it certainly cannot be if it be associated with the graptolites occurring in the strata throughout which *Tetragraptus* ranges. The fact that certain species appear to be restricted to definite horizons of these *Tetragraptus* beds is of interest, for until quite lately the existence of definite zones in the corresponding Skiddaw and Quebec beds, both of which have undergone much disturbance, was doubted.

Consideration of the contributions to our knowledge of the Upper Palæozoic or Deuterozoic (Devonian, Carboniferous, and Permian) beds, which were made during the second half of the year 1894, must be left for a future article.

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ON THE RESPIRATORY FUNCTION OF STOMATA.

THE part played by the stomata of a leaf in the gaseous exchanges essential to respiration and assimilation has been much debated, and at the present time leading text-books express entirely opposed views. Yet it would seem to be a question of primary biological importance whether the vast quantities of carbon dioxide that are taken up by assimilating green leaves enter through the stomatic openings or are absorbed by the much more extensive, cuticularised, exposed surfaces of the epidermal cells. Until this is decided no very definite conception of the physiological significance of the leaf structure is possible.

It is proposed to give in this article, firstly, an historical sketch of the conflicting theories and experiments that have been advanced in the attacks on this problem; then a brief account of a complicated apparatus that the author has designed, capable of obtaining direct experimental evidence from the living leaf on this function; and finally a *résumé* of the results so obtained, which it is trusted furnish a final solution of the problem.

The earliest paper that must be noticed is by Dutrochet (1), 1832. He draws attention to the extensive system of intercellular spaces to be found in leaves, especially in the lower strata, and demonstrates by injection the connection with the stomata. From determinations of the composition of the gas normally contained within these spaces he concludes that this internal air has a real physiological significance, and that the leaf of the plant has a lung-like function, handing on to the outer air the carbonic acid formed within the bulk of the plant, and returning oxygen in exchange.

Interest in the *rôle* played by cuticle, then newly investigated, in absorption and exhalation by the aerial parts of the plant, stimulated Garreau (2) in 1850 to carry out a series of researches on these points.

Among other results he arrived at the well-known one that transpiration of water-vapour takes place to a much greater degree through areas provided with stomata than through uninterrupted cuticle, so that more water is given off from the lower than from the upper surface in the great majority of leaves.

In the same paper in which these often-quoted experiments are detailed, there is a usually neglected section which bears immediately on the subject now under consideration. In this Garreau gives the results of the extension of his method to the comparison of the amounts of carbon dioxide given off by the two surfaces of leaves respectively.

He attached a glass chamber containing a small shallow capsule of lime water to corresponding areas on each surface of a leaf. The carbon dioxide liberated by the leaf produced a film of calcium carbonate, of varying thickness, on the surface of the lime water; this was too fragile for direct weighing, so he collected it on pieces of tared filter-paper, and after drying determined the gain in weight. His estimations are all too high, probably increased by CO_2 from the air, and the method is of course decidedly inadequate; but the results tended strongly to show some relation between the distribution of stomata and the evolution of carbon dioxide, and, among the conclusions to the paper, we find it stated that the expired quantities of carbon dioxide appear to be in a much closer relation to the distribution of stomata than do those of exhaled water. No attempt to obtain similar numbers has been published during the time that has elapsed between the paper under consideration and the present author's work.

In 1867, Boussingault contributed a long experimental paper (3). He directed his attention entirely to the gaseous exchange taking place during active assimilation. As this exchange far exceeds in intensity that of respiration, the former subject appears much the more likely to give a decisive experimental answer to the problem of the nature of the path of exchange.

The results, however, led him to take up a view exactly

the contrary of the one supported by Garreau, and to minimise the function of stomata in gaseous exchange. He appeared to prove by trustworthy experiments that the vigorous intake of carbon dioxide during assimilation in bright light proceeded chiefly through the astomatic cuticle. These experiments demand experimental criticism, which must be reserved until the historical survey is complete. It may be here pointed out that his critical experimental proof consisted in showing of two otherwise similar leaves, that the one which had its stomata mechanically blocked did not decompose any less carbon dioxide than the leaf with its stomata in open functionable condition.

Barthélemy (4) published his first paper on this subject in 1868. After premising on the strength of Boussingault's researches that stomata only play a secondary *rôle* in gaseous exchange, and that there exists no fixed relation between number or size of stomata and assimilatory activity, he brings forward for the first time the view that the cuticle plays a definite specific part in gaseous interchange. This view was suggested to him by Graham's (5) work on the osmosis of gases through thin films of caoutchouc combined with Frémy's (6) then accepted determination of the chemical constitution of cuticle, as being very similar to that of caoutchouc. Carbon dioxide is transmitted through caoutchouc with a rapidity far exceeding that of other ordinary gases, and, of course, it is this gas that must make its way with great rapidity to the internal cells of the leaf during assimilation. Cuticle, then, according to Barthélemy, is to be regarded as a membrane specially fitted by its physical colloid properties to transmit carbon dioxide into the interior of the leaf. Nothing is suggested as to the path by which the equally large quantities of oxygen formed are passed out of the leaf.

In support of his theory he adduced experiments on the osmosis of gases through leaves, which yielded him numbers almost the same as those obtained for caoutchouc by Graham. The experiments are, however, absolutely valueless. CO_2, he concludes, enters leaves almost entirely through the cuticle. As for the stomata, he enunciates the view that

they act as valves, allowing gases to pass out when the internal pressure is high.

In 1877 and 1878, Merget contributed three short notes to the French Academy opposing Barthélemy, and experimentally supporting the stomatic *rôle* by ingenious though rather inconclusive diffusion experiments.

The first (7) attempts to follow the path of diffusion into leaves of those vapours whose passage can be traced by their discoloration effects. Of these, mercury vapour is considered to yield the most decisive results, as it is said that it cannot be carried in a water current nor osmose through a colloid membrane. Boussingault (8) and others have shown the strong toxic action of this vapour, and that it particularly attacks protoplasm, producing dark discoloration and death. If a leaf be exposed to mercury vapour, even the deeply situated cells turn dark in a short time. From this ready penetration by a substance that cannot osmose, Merget argues that the vapour must pass through the stomata and penetrate by the intercellular spaces. Also, if a leaf be injected with mercury and pressed between paper sensitised with $AgNO_3$, the blackening of the paper by the vapour only occurs in those parts which bear stomata. The interpretation given is no doubt correct, but it is not logically conclusive, for the very fact that mercury attacks protoplasm shows that it must osmose through a colloid membrane—the cell wall—and so might enter the leaf partly through the cuticle. One fact the author does establish, namely, that diffusion takes place through stomata with equal facility in either direction. Several short controversial papers by Barthélemy and Merget then appeared without much changing their original positions.

No further papers bearing directly on this subject appeared till 1887, when Mangin published two summary notes (9), (10), and then followed them up in 1888 by a detailed paper (11) of careful experiments on diffusion through cuticle.

The fundamental object of this paper is the determination, firstly, of the quantity of gas that might diffuse under

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given conditions across the isolated cuticle; and secondly, of the quantity of this gas absorbed or exhaled by the organ to which the cuticle belongs, in full activity. If the former is less than the latter, stomata must come into use, but if the former is the greater, then osmosis may be sufficient to account for all the exchange.

The diffusion experiments are carried on in an ingenious apparatus invented by the author, in which the cuticle under investigation serves as a diaphragm between two chambers, one of which is kept filled with CO_2, while the other is closed, contains some other gas, and is connected with an adjustable manometer, the movements of which record the amount of gas diffusing into or out of the chamber through the diaphragm of cuticle.

The method appears excellent, but the materials used seem to be open to objections. The cuticle of various leaves is isolated, by allowing the leaf to remain macerating for some weeks in a culture of *Bacillus Amylobacter*, which gradually destroys the rest of the tissues. Pieces of cuticle, two to four centims. square, are thus obtained and employed. In order to render them more resistant, they are painted over with a 10 per cent. warm solution of glycerine jelly. Of this it is alleged that it produced no appreciable increase of the resistance of the cuticle to the passage of gases, and that it will serve to block up the stomata and cracks which have hitherto been the great stumbling-block in such diffusion experiments. Surely it must still be objected, that, since it is so permeable, gas will pass much quicker through those spots where there is nothing but a film of glycerine jelly covering a stoma or crack than through the unperforated cuticle. The osmosis numbers obtained one would expect to be much too high. Thus, prepared cuticle gives numbers for the rates of diffusion of equal volumes of different gases agreeing closely with those obtained by Graham for caoutchouc.

Fallacious as may be the absolute numbers, the relations between different cuticles are of interest. The cuticle of the lower surface of the leaf is always found to be more permeable than that of the upper, but never more than five

times, in spite of the fact that the lower is usually riddled with stomata covered only with a film of glycerine jelly, while there are but few of these weak spots on the upper cuticle. This shows that the actual permeability of the cuticle of the lower surface, apart from the stomata, is never as great as five times that of the upper cuticle, a determination that we shall subsequently see to be of importance.

In the second part of his paper, from experiments on the respiration and assimilation of leaves, the author comes to the conclusion that the amount of osmosis that could take place through the cuticle, as calculated from the above data, is quantitatively insufficient to account for the whole of the necessary gas exchange, but may account for a half to two-thirds of it in respiration. During assimilation, however, he holds that the stomata must carry on practically the whole of the exchange as the tension of CO_2 in the air is too small to produce much osmosis through the cuticle.

In a note to a paper on starch formation from sugar and other substances, Boehm, in 1889 (12), mentions that he has performed experiments which go to prove the prepondering *rôle* of stomata in the gas exchange of assimilation. He exposed thick hypostomatic leaves, laid with their lower faces on the surface of water, to bright light in an atmosphere rich in carbon dioxide, and found that starch was only formed in small areas. He concludes that gases diffuse only extremely slowly through the epidermis cells, and the stomatic path of gaseous exchange must be much the more important.

The most recent work on our subject is by Stahl, 1894 (13), who has anticipated myself in publication of one class of experiments that go to prove the almost absolute *rôle* of stomata in assimilatory exchange.

These consist in showing the local absence of starch formation when a limited area of a leaf is coated on its stomatiferous side with some substance that mechanically blocks the stomata. This forms an easy demonstration experiment; Stahl tried it on numerous leaves, with the uniform result that, while starch was plentifully formed in normal parts of the leaf, no starch appeared in the coated parts after long exposure to light.

The chief part of my own work (20) on this subject was carried out in 1893 and the whole of it before Stahl's paper appeared. At that time the only piece of experimental work, among those given above, that was held to be above suspicion or explanation was that done by Boussingault, and on this elementary teaching, at all events in England, has been hitherto based. Feeling the need for direct determinations of the intake and output of CO_2 by stomatic and astomatic leaf areas respectively, and finding all the recognised methods of estimation insufficient for my purpose, I commenced, keeping specially in view the needs of biological research, to simplify the technique and improve the delicacy of the one which offers the greatest potentiality of accuracy, namely, the volumetric estimation of the CO_2 by standard solutions of baryta and of an acid. After more than a year's work I have succeeded in designing an apparatus of great chemical delicacy and specially adapted to biological work.

Thus by its aid the evolution of CO_2 by a small area of a foliage leaf can be estimated from hour to hour without a break, for any desired time, while for the same area of leaf, the more active absorption of CO_2 in assimilation can be easily determined for such short periods of time as fifteen minutes, and that at the same time separately for the two surfaces of one and the same leaf area. Further, for the purposes of this assimilation, a current of air containing any desired proportion of CO_2, however small, can be supplied continuously to the tissue under investigation. Moreover, strictly comparable experiments can be carried on simultaneously as the apparatus is practically in duplicate throughout.

The actual estimation of the CO_2 is accomplished by the well-known method of absorption by baryta solution and titration with hydrochloric acid. The novelty consists in this, that only a very small quantity of baryta solution (under 15 c.c.) is employed in each experiment, and that after the absorption the whole of this is titrated with acid in the tube in which the absorption has taken place. Further, the burettes containing the standard solutions are always in air-tight connection with this absorption chamber,

and no air beyond the current under investigation is ever admitted to the chamber, except such as has been carefully freed from CO_2. The special arrangements for stirring and emptying, by means of this air, freed from CO_2, and stored under pressure, cannot be entered into here.

The two currents of air passing continuously through the apparatus are generated by two aspirators of a special type, which, worked on the principle of Mariotte's bottle, gave a practically constant outflow in drops, whatever the level of the water within them, and are adapted to work steadily with small rates of flow (50 to 100 c.c. per hour). These currents enter the apparatus, either through an arrangement for removing the CO_2 when working on respiration, or when working on assimilation through one for adding CO_2. Both these are so constructed that the current never has to bubble through a layer of liquid, and so is supplied to the plant at strictly atmospheric pressure, thus avoiding any risk of drawing gases mechanically out of the part under investigation. The remover of CO_2 is a "tower" full of beads, over which a stream of strong potash flows continuously, and through which the air is drawn. The CO_2 generator is constructed on a new principle, and consists of a tall tube containing fragments of marble, through which the air current passes at a constant rate, while very dilute HCl trickles down it at an extremely slow constant rate. Thus a constant amount of CO_2 is being continually generated and is carried off by the air current. The amount of CO_2 formed can be controlled by the strength of the acid employed. When generating amounts below 2 per cent. of the air current, this arrangement works very satisfactorily. From the CO_2 generator or remover, as the case may be, the current of air passes to the receivers, in which the parts of the plant under investigation are situated. These receivers are of various forms, according to the material experimented on, but are all constructed on the cardinal principle of making them as small as possible consistent with the well-being of the part, in order that changes in the composition of the gas shall, as soon as possible, be felt by the current which passes thence through narrow tubes to

the absorption chambers. When titrations are being made, and the air current can no longer be allowed to pass through the absorption chambers, it passes through a column of water equal in its resistance to that of the baryta solution in the absorption chamber. This enables the rate of flow to be kept constant between, as well as during, the actual experiments. Numerous other details, such as the special method of refilling the burettes, etc., and above all those small points by which constancy is, as far as possible, attained, many of which have involved weeks of special experiment, cannot be described here.

Simplifications of technique by complication of apparatus has been the guiding principle, and the result is that, although the whole consists of at least eight separate pieces of apparatus, many being further in duplicate, and all connected together by a plexus of tubes, yet the working is so automatically arranged that the operator, beyond reading the burettes and occasionally working a finger bellows, has nothing to do but turn stopcocks.

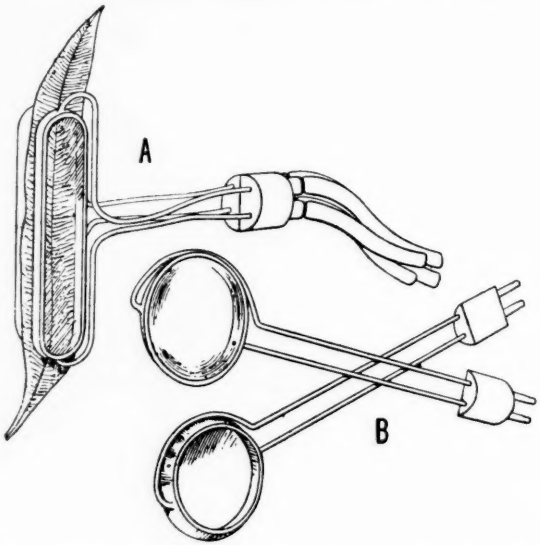
If only one series of estimations is being made, these can be kept absolutely consecutive, the current being led through one of the absorption chambers, while the solution in the other one is being titrated and renewed, and so on alternately. When two series of comparative estimations are being made at once, a small interval must be allowed after each double estimation, during which the titrations are performed; the currents of air in connection with the plants then pass through by-paths, still at their previous rate.

Delicacy of estimation sufficient for present work is obtained by the use of half-decinormal, N/20, standard solutions. Phenolphthalein is used as indicator, and specially delicate end-reactions can be obtained, since atmospheric CO_2 is excluded, and moreover the burettes containing *both* the solutions can be drawn upon.

Series of control titrations often give an error of observation not exceeding 1/200 c.c. of CO_2, and 1/50 c.c. is sufficient for a trustworthy estimation in experiments of short duration.

ON THE PATHS OF EXIT OF CARBONIC ACID FROM LEAVES.

Aided by this apparatus the author has been able to carry out direct experiments on the two surfaces of living leaves, and to determine what relation there is between the distribution of the stomata and the amounts of CO_2 exhaled in the dark or absorbed in bright light. In order to differentiate between the two surfaces, the chambers figured below have been designed. Two different pairs are represented.



Each consists of a brass rim permanently closed on the one side by a glass plate, and attached hermetically on the other side to the surface of the leaf by a little wax, which manipulation can easily be accomplished with the aid of a hot wire. The rim is pierced by two fine metal tubes which also pass through hemi-cylindrical blocks of brass by which the chambers can be clamped together in their right positions on the two surfaces of a leaf.

Two continuous slow currents of air generated by the aspirators are then kept up, through these chambers, over either surface of the leaf. In experiments on respiratory exhalation, the current is freed from CO_2 by the potash "tower" before it enters the chamber. Leaving the

chamber, it passes to the apparatus where the CO_2 it has taken up is absorbed by the standardised baryta. At the end of the experiment, without interrupting the current, the amount of baryta unneutralised is estimated with the standard acid, and thence the amount of CO_2 liberated during that experiment easily calculated.

The area of leaf covered by each capsule is just ten sq. cms., and as the amount of CO_2 given out by surfaces poor in stomata is very small, the experiments on this point are often of twelve hours' duration.

In the appended table of results the amounts of CO_2 evolved, given in the third and fourth columns, are expressed in cub. centims. per hour and ten sq. cms. It is obvious that there is a close general agreement between the distribution of stomata and the exhalation of CO_2.

Plant.	Stomatic Ratio for the two Surfaces.	Upper Surface.	Lower Surface.	Ratio of Amounts of CO_2.
Nerium oleander .	$\frac{0}{8}$	0·002	0·078	$\frac{3}{100}$
Hedera helix . .	$\frac{0}{8}$	0·002	0·054	$\frac{4}{100}$
Platanus occidentalis .	$\frac{0}{8}$	0·001	0·050	$\frac{2}{100}$
Ampelopsis hederacea	$\frac{0}{8}$	0·003	0·100	$\frac{3}{100}$
Alisma plantago .	$\frac{135}{100}$	0·030	0·025	$\frac{120}{100}$
Iris germanica . .	$\frac{100}{100}$	0·029	0·026	$\frac{110}{100}$
Ricinus communis leaf . . .	$\frac{40}{100}$	0·015	0·036	$\frac{40}{100}$
Ricinus communis cotyledon . .	$\frac{70}{100}$	0·012	0·022	$\frac{55}{100}$
Populus nigra . .	$\frac{18}{100}$	0·010	0·037	$\frac{26}{100}$

The very small amount estimated for the upper surfaces of those leaves which are said to have no stomata above, must be partly made up of the CO_2 passing through the occasional isolated stomata which are known to occur in such cases, and partly of the minute amount of CO_2 which

inevitably leaks or diffuses in from the external air during an experiment of twelve hours' duration.

Allowing for these increments, it may well be that through an absolutely uninterrupted cuticle, no CO_2 at all diffuses if there be an open alternative route through the stomata. We are certainly justified in concluding that the stomata are *practically* the sole path for exhalation of respiratory CO_2.

ON THE PATH BY WHICH CARBON DIOXIDE PASSES INTO LEAVES DURING ASSIMILATION.

The experiments on this point are conducted on essentially the same plan as those on respiration, with the difference that the air coming into the capsules is not freed from CO_2, but is loaded with a definite percentage of this gas by the "constant CO_2 generator".

After the leaf has been carefully fitted up in the capsules figured above with the inlet tubes for each side attached to the CO_2 generator and the outlet tubes to the apparatus, the aspirators are allowed to run for a couple of preliminary hours that the strength of CO_2 may attain constancy. During this time the leaf is kept completely darkened, but must be so arranged that when the covering is removed a bright light falls on the capsules at right angles to the glass side so as to fully illuminate the leaf within. At the end of this time an estimation of the CO_2 passing over either surface is taken for say fifteen minutes, and then the leaf may be illuminated. After a certain length of illumination, a new estimation of the CO_2 is made for the same length of time as before. If any CO_2 has been taken up by either surface of the leaf, the current from that chamber will show a falling-off in its CO_2 content; and, if it does not show this, then no CO_2 has been taken up. Subsequently, the illumination may be altered or stopped, and, after a short time, another estimation made, and so on, ending with a pair of estimations in darkness, when, if all has gone well, the original amounts of CO_2 should be again obtained.

The details of two experiments are appended; the first with a leaf of *Alisma Plantago* which has rather more stomata above than below.

No.	Conditions of Illumination.	Baryta solution neutralised.		CO_2 absorbed by the leaf.	
		Upper surface.	Lower surface.	Upper surface.	Lower surface.
		cub. centim.	cub. centim.	cub. centim.	cub. centim.
1	Dark, 11·45 to 2·5 Estimation, 1·50 to 2·5 . . .	0·30	0·28	0·00	0·00
2	Sun through ground glass, 2·5 to 3·5 Estimation, 2·20 to 2·35 . . .	0·09	0·17	0·10	0·06
3	Sun, as above. Estimation, 2·50 to 3·5	0·00	0·07	0·15	0·11
4	Sun through thin paper, 3·5 to 3·35 Estimation, 3·20 to 3·35 . . .	0·10	0·14	0·10	0·07
5	Shaded on the upper surface; sun on the lower surface, 3·35 to 4·5 Estimation, 3·50 to 4·5 . . .	0·15	0·14	0·07	0·07
6	Dark. Estimation, 5·5 to 5·20 . .	0·28	0·28	0·00	0·00

The baryta solution employed is of such a strength as to unite with just half its volume of CO_2. By this the amounts of CO_2 absorbed by the two surfaces of the leaf, as given in the fifth and sixth columns, are calculated from the falling-off in the amounts of baryta that the CO_2 left unabsorbed is able to neutralise.

The assimilation in this experiment is very vigorous; in reading three, the whole of the CO_2 supplied to the upper surface [0·6 per cent. of the air current] is taken up. The intake is nearly equal on both surfaces of the leaf.

Contrast with this a second experiment made on a leaf of *Ampelopsis hederacea* which bears all its stomata on the lower surface.

No.	Conditions of Illumination.	Baryta solution neutralised.		CO_2 absorbed by the leaf.	
		Upper surface.	Lower surface.	Upper surface.	Lower surface.
		cub. centim.	cub. centim.	cub. centim.	cub. centim.
1	Dark from 2·30 to 5·19 Estimation, 5·4 to 5·19 . . .	0·74	0·76	0·00	0·00
2	Diffuse light, 5·19 to 6·5 Estimation, 5·50 to 6·5 . . .	0·74	0·68	0·00	0·04
3	Sunlight (feeble), 6·5 to 6·32 Estimation, 6·17 to 6·32 . .	0·74	0·48	0·00	0·14
4	Dark. Estimation, 7·34 to 7·49 .	0·76	0·78	0·00	0·00

In this case even in direct sunlight no CO_2 is absorbed by the upper surface of the leaf; all that is taken up enters on the lower surface.

From the combined consideration of the concordant results obtained on respiration and assimilation, we see definitely that, at least, as far as carbon dioxide is concerned, gaseous exchange closely follows stomatic distribution on the two surfaces of leaves. From this it results that this exchange takes place practically entirely through the openings of the stomata into the intercellular spaces. The only alternative hypothetical possibility, that in every case the exchange takes place across the epidermis cells and cuticle of those *areas which bear stomata*, and yet not through the stomatal openings, can hardly be seriously entertained. This supposition would involve, among others, the assumption that the cuticle on the lower surface of hypostomatiferous leaves is often fifty to a hundred times more permeable than that on the upper surface. Mangin has however shown that it is never more than four or five times as permeable. The normal difference in gaseous exchange, which is almost of a higher order of magnitude, cannot therefore possibly be explained in this way.

It is further of interest to note the assimilation by the leaf in bright light of its own CO_2 formed by respiration. An experiment was made with a leaf of *Acer platanoides* (all the stomata below) by attaching a capsule to the lower surface only, and supplying to it a current of air freed from CO_2.

No.	Conditions of Illumination.	Total CO_2 escaping.	CO_2 per hour.
		cub. centim.	cub. centim.
1	Direct sunshine, 11·6 to 3·6		
	Estimation, 12·6 to 3·6 . . .	0·00	0·00
2	Dark, 3·6 P.M. to 5·35 P.M.		
	Estimation, 3·55 to 5·35. . .	0·135	0·08
3	Diffuse light, 5·35 to 8·1		
	Estimation, 5·46 to 8·1	0·04	0·018

The third estimation was however commenced rather too soon after the period of darkness for this to be quite conclusive evidence of escape of CO_2 in diffuse light.

In the second reading we have the CO_2 of respiration made evident, but this in the first reading is wholly assimilated.

This and other similar estimations show conclusively that the classical experiment by Garreau (14) demonstrating the liberation of carbon dioxide in bright light is misleading. That a little CO_2 continually escapes from an entire branch put in a closed chamber with baryta water in the sun is not to be wondered at. The shaded parts of assimilating tissues, together with the non-assimilating tissues of petioles, stems, buds, etc., might quite account for this, but it is difficult to see how any carbonic acid should escape from a completely illuminated leaf surface of normal assimilatory activity. The result of Garreau's experiment is then only an expression of the uncritical conditions in which it was made. Corenwinder (15) showed in 1878 that with young but expanded leaves the chlorophyll of which was not yet fully developed, an evolution of carbon dioxide takes place in the sun, and that this is clearly due merely to the imperfect development of the assimilatory function, for the amount of the CO_2 liberated increases in the dark. With mature leaves he found that no liberation of CO_2 takes place in sunshine.

ON THE GASEOUS EXCHANGE IN LEAVES WHEN THE STOMATA ARE OCCLUDED.

As I have stated above Boussingault has been generally held to have proved that during assimilation CO_2 is taken up chiefly by the epidermis cells of the upper leaf surface which directly adjoin the assimilating palisade cells. He arrived at this conclusion by taking two similar leaves of Oleander, coating the upper surface of one and the under surface of the other with lard, and then exposing them to light in vessels containing known amounts of CO_2 and air. He found that whereas the one assimilated 10 c.c. of CO_2 the other with its stomata blocked assimilated 17·5 c.c. He drew the obvious conclusion that the upper stomata-less surface is the chief seat of absorption and that it is the coating of this with lard that reduces the assimilation to 10 c.c. of CO_2 in the first case.

For some time after I proved, by the direct experiment described in the previous section, that the absorption does not take place through the astomatic surface, this result of his was an enigma to me.

That the cuticle of leaves was slightly permeable to CO_2 was well known, but how was one to explain the greater intake of CO_2 in the leaf with its stomata blocked than in the leaf with its stomata open? It occurred to me that the explanation might lie in the large CO_2 content of the gas in which Boussingault exposed the leaves. The CO_2 formed more than 30 per cent. of the mixture, and the less decomposition of CO_2 in the leaf with stomata open might be due to its receiving not *less* CO_2 than the other leaf, but *more*, indeed so much more above the optimum for assimilation that it decomposed much less than the leaf inside which the CO_2 was not so abundant. Within the latter, CO_2 would never become very plentiful, diffusing slowly as it must through the upper surface of the leaf, as the stomata were blocked with lard. I then turned to Godlewski's well-known paper (16) on the optimum strength of CO_2 for assimilation, and found that he had worked on this same leaf, *Nerium*, and that although his results are not very decisive, there was evidence that the Boussingault result could be interpreted in this way. Thus Godlewski found the optimum strength of CO_2 for assimilation in *Nerium* to be decidedly lower than the 8 per cent. which is the number for other leaves. On looking through his experiments with *Nerium* there will be found one, No. xxxiv., which just seems to fit the case in question. In this experiment he exposed two leaves to the light in 28 per cent. CO_2 and 3·6 per cent. CO_2 respectively, and the amounts of CO_2 decomposed were as 10 : 17·8. Assume that from an environment of 30 per cent. CO_2, the CO_2 diffuses through the cuticle of the leaf with blocked stomata at such a slow rate that there is only as much CO_2 inside the leaf as would result from about 3·6 per cent. outside were the stomata open, and the whole contradiction is gone.

The truth of the above explanation of Boussingault's result will be confirmed if it be shown that in an atmosphere which contains an amount of CO_2 that is but little, if at all,

above the optimum for assimilation, the reverse result is obtained and the leaf with open stomata decomposes more CO_2 than the one with the stomata blocked. I have, therefore, performed a series of experiments similar to Boussingault's, but with leaves of *Nerium* in varying percentages of CO_2, and have found that in a high percentage the leaf with its stomata blocked always decomposes more than the normal leaf, while in low percentages it decomposes less.

These experiments were all performed, with slight modification, by the method brought to perfection by Pfeffer, and described in detail with all its possible sources of error in his paper, "Die Wirkung farbigen Lichtes auf die Zersetzung der Kohlensäure in Pflanzen" (17).

I append a synopsis of the experiments arranged according to the strengths of CO_2 in which they were carried out.

While in 6 per cent. CO_2 the leaf with blocked stomata decomposes only one-seventh as much as the normal leaf, in 50 per cent. CO_2 the relation is completely reversed and it decomposes one and a half times as much.

Mean percentage of CO_2 present in each experiment.	Absolute amounts of CO_2 decomposed per sq. cm. of leaf surface.		Relative amounts of CO_2 decomposed by the leaves with blocked stomata, that of the normal leaves being taken as unity.
	Normal leaf.	Vaselined leaf.	
	cub. centim.	cub. centim.	
6	0·07	0·01	0·14
6·3	0·055	0·01	0·20
7·5	0·046	0·017	0·21
14	0·18	0·04	0·37
55	0·049	0·067	1·3
50	0·043	0·069	1·5
97	0·033	0·060	1·8

Boussingault's work then, thus explained, ceases to support the cuticular exchange theory and yields but a confirmation of the view that the stomata offer a far easier entrance for gases into the leaf.

A due consideration of the differences in assimilation between leaves with stomata open and leaves with stomata blocked brings out the relativity of that which we speak of as the *optimum* percentage of CO_2 for assimilation. The

numbers experimentally obtained for this function really express the *external optimum* of carbon dioxide content—a number very convenient to know, but not of direct physiological significance. The *internal optimum* for the atmosphere surrounding the assimilating cells themselves must be a smaller number, which might well be constant for all cases. The external optimal number would then be a combined expression of this constant and a variable, which would depend on the structure of the leaf in question, and would be large for those leaves which, by reason either of the smallest or scarcity of their stomata, the narrowness of their intercellular spaces or the thickness of the leaf, offered considerable resistance to the diffusion of carbon dioxide.

How variable the size and numbers of stomata may be, the determinations given by Weiss (18) plentifully exhibit, and Unger (19) has shown that the volume of the intercellular spaces in leaves may vary from 3 per cent. to 71 per cent. of the total volume of the leaf. Whether all this variation really does produce a marked effect on the penetrability of leaves we cannot yet say.

As a corollary to the experiments just given, mention must be made of the effect of exposing leaves that have their stomata blocked with vaseline to so small a percentage of carbon dioxide as that occurring in the atmosphere. Here the difference between the vaselined leaf and the normal leaf is very marked, and, according to the usual phraseology, the vaselined leaf does not assimilate at all, that is to say, it forms no starch on exposure to bright light.

If a leaf with its stomata all on the lower surface be freed from starch by darkness, and then, with half the under surface coated with vaseline, be exposed for a whole day in a good light, no starch will be found in this half, while the other has formed starch freely. Coating the upper surface has no effect on starch formation. I performed such experiments in the summer of 1893 and showed them to several botanists. Nearly at the close of my research, in July, 1894, Stahl (13) published similar experiments. He found a mixture of cocoa-butter and bees'-wax preferable to vaseline. His observations are much more extended than mine, and he gives some interesting figures of the starch

formation that takes place round wounds or openings in the upper epidermis under these circumstances. I obtained the best results with leaves of *Vitis*, *Polygonum sacchalinense*, and especially with *Sparmannia*. In its suitability for demonstration purposes this experiment should be of great service in dispelling the established English view as to the paths of gaseous exchange.

In conclusion it must be noted that throughout only the carbon dioxide of the gaseous exchange and not the oxygen has been localised. Chemical analysis hardly permits us to attempt any such work with this latter gas. It can, however, hardly be doubted that its movement is normally limited to the stomata as much as that of carbon dioxide. For, on the one hand, it must theoretically diffuse more readily through small openings such as stomata, since its density is less, and, on the other hand, it has been experimentally proved to pass through cuticle less readily than carbon dioxide.

The passage of CO_2 into the leaf during assimilation is the most difficult and crucial of the four movements of gases, two of respiration and two of assimilation, that take place. In any of the other three the differences of tension between the inside and outside of the leaf may increase to almost any extent, while for this case the difference of tension never exceeds 0·3 millim. Hg., and the cuticle of land plants is practically impenetrable to this small tension.

The internal structure of leaves is generally in accordance with the view that all gaseous exchange takes place through stomata, for the intercellular spaces narrow towards the palisade cells, forming a sort of funnel with the wide end at the stomatal region. The active cause of the divorce which results in the palisade cells and stomata, whose united action is necessary for vigorous assimilation, being usually found on opposite surfaces of the leaf, is presumably the double necessity for at once limiting transpiration and illuminating the assimilating cells.

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THE ZOOLOGICAL POSITION OF THE TRILOBITES.

THE veil of mystery which has so long hung over these early inhabitants of the earth has, within the last few months, been almost completely withdrawn. The apparently endless variations of the Trilobites, none departing far from the common type, have so long excited the admiration of intelligent students of nature, and have given rise to so many speculations as to their relationships, that an account of the most recent discoveries can hardly fail to be welcome to a wide circle of readers.

The fact that only the dorsal surfaces of the Trilobites have been, as a rule, preserved, has always been especially tantalising. While few natural objects have been so closely and exhaustively studied as the jointed dorsal shields of the Trilobites, their under surfaces have hitherto eluded even the most searching investigations. Beyond the great labrum, very little could be found which with any certainty could be ascribed to the under surface of the original animal. It is this long-looked-for under surface which has now been partially discovered in at least two species.

Although the new light has burst thus suddenly upon us, the patient researches of naturalists, like Henry Woodward and Walcott in recent times, not to mention the earlier workers, have not gone altogether unrewarded. Fragmentary glimpses of limbs, parts of limbs, and even parts of series of limbs have been brought to light. But so dense has been the mystery enveloping the group, so easy was it to find traces which might be limbs or might not, that all "discoveries" of this kind were looked upon with suspicion.

The limbs have now at last been discovered in a way which leaves little to be desired, and it is now possible to assign to the Trilobites their real position in the scale of animate nature with an accuracy for which, two years back, no one would have dared to hope. It must not, however,

for a moment be supposed that we know all about the Trilobites—far from it. In spite of the new and splendid discoveries in America, our knowledge remains very fragmentary. But among the additional facts which are now in our possession are just those which were most wanted by the morphologist to enable him to give the Trilobites their true place in the animal kingdom.

As I propose to pass these new facts in review and to discuss their bearings, a longer retrospect must be dispensed with. Suffice it to say that until comparatively recently zoologists, relying upon comparisons with existing groups, were generally agreed that the Trilobites were in some way related to the existing Crustaceans, but as to the nature of the affinity opinions greatly differed. In 1881, however, Professor Lankester[1] connected them with the Arachnids rather than with the Crustacea, basing his argument upon an elaborate comparison of *Limulus* and certain *Eurypterids* (both of which are undoubtedly related to the Trilobites) with *Scorpio*. This suggestion is still a matter of controversy, and a review of the arguments for and against would take us too far from our present subject. Further, the Trilobites, in spite of their prime importance in all discussions as to the affinity of the Merostomata, play only a passive part in the discussion, being dragged in, as if of small account, by their specialised offshoot *Limulus*. We may, however, at once state that the new evidence as to the position of the Trilobites is entirely in favour of the older view which linked them with the Crustacea.

Returning then to this older hypothesis, two views were sufficiently clearly defined to admit of discussion and criticism. One sought to ally the Trilobites with the Phyllopods, and relied chiefly upon the variability in the segmentation common to these two, and also upon the likeness between the head shield of *Apus* and of other extinct Phyllopods with the head shield of the Trilobites. The other saw in the Trilobites the ancestors of the modern Isopods, and relied chiefly upon the great external resemblance existing between the two forms. Neither case was really very strong. The absence in the

[1] "*Limulus* an Arachnid," *Q. J. M. S.*, vol. xxi.

Phyllopods of anything like the pleuræ of the Trilobites, and the difference between the leaf-like limbs of the former and the filamentous limbs attributed to the latter, were acknowledged difficulties against the former supposition. While on the other hand, the fixed segmentation of the Isopods and their uniform division into well-defined head, thoracic, and abdominal regions showed them to be far more highly organised than the Trilobites. Lastly, against both these affinities the total absence of any traces of antennæ in the Trilobites served as a warning against any too rash assertion of their Crustacean affinities.

So far, however, as this absence of the antennæ in the Trilobites could tell us anything, the advocates of the Phyllopodan affinity were better off than the advocates of the Isopodan affinity. In the Phyllopod *Apus*, the antennæ are not visible from the dorsal surface, being ventrally placed. If the antennæ in the Trilobites were like those of *Apus*, they would have vanished with the rest of the ventral appendages. Their absence in the Trilobites therefore is just what their assumed relationship with *Apus* would lead us to expect. On the other hand, the same fact was a serious difficulty against the Isopod relationship, as these have very pronounced, anteriorly-placed antennæ, which made it difficult to understand how they could be completely absent in the Trilobites, if there were any genetic relationship between them.

In 1892 the present writer made a detailed study of the morphology of the Phyllopod *Apus*, which led him to the conclusion that it was the most primitive of all existing Crustacea, and was, indeed, an ideal transition form between the chaetopod Annelids and the higher Crustacea. Such a conclusion necessarily led to a comparison between *Apus* and the earliest known fossils which had any claim to be considered Crustacea, *viz.*, the Trilobites. Several new points of resemblance were discovered, of which one was of prime morphological importance. The most startling feature in the organisation of *Apus* was shown to occur also in the Trilobites.

The peculiarity referred to is the remarkable character

of the system of appendages in *Apus*. They commence anteriorly, one well developed pair on each segment. But from before backward they progressively diminish in size, and about half-way along the trunk commence to be more and more crowded. The most posterior pairs are quite microscopical, and so crowded are they that as many as six pairs appear to occur on one and the same body segment. There are occasionally as many as sixty to sixty-five pairs of appendages in all! This phenomenon is quite unique among existing Arthropods, and had long appeared inexplicable. The explanation offered by the present writer was briefly as follows :—

Apus, as the most primitive of all extant Crustaceans, had for its immediate ancestors animals with some sixty to seventy developed segments. In *Apus* these sixty or more inherited segments commence to appear, but the bulk of them remain rudimentary. We thus have the most developed limbs anteriorly, while posteriorly they decrease in size until they are little more than closely crowded limb-buds. Each pair of limbs, therefore, represents a true segment. In the posterior trunk region of *Apus*, where more than one pair of limbs occurs on a body ring, this latter is not a true segment, but a secondarily formed segment, consisting morphologically of as many true segments as there are pairs of limbs developing from it.

This explanation was strongly confirmed by an examination of the internal organisation of *Apus*, in which the progressive rudimentation from front to back which it postulated could be clearly traced, notably in the arrangement of the musculature in the heart, and in the genital glands. On the other hand, that each limb-bud represented a true segment seemed to be established by the presence of a distinct ventral ganglion for each pair. Lastly, an appeal to the developmental history left little doubt as to the correctness of our interpretation of this long-standing enigma. Allowing for special modifications of the anterior functional appendages of the *Nauplius*, it is, at any stage, merely a small *Apus* showing the same phenomenon. The anterior functional limbs are followed by a series of

gradually diminishing rudimentary appendages. The fully developed *Apus* is, in fact, little else than an enlarged, not metamorphosed, *Apus Nauplius*. At any stage at which the growth stopped there would always be a number of progressively diminishing limb-buds and undeveloped segments. We accordingly find in the different species of *Apus* a very varying number of these rudimentary limbs behind a certain number of well-developed appendages at the anterior end of the body.

This argument is still further strengthened when the character of the limbs is also taken into account. A distinct tendency towards the formation of jointed filamentous appendages can be seen at the anterior end of the trunk. This is gradually lost towards the posterior end, and the limbs become more and more leaf-like and primitive, *i.e.*, they are flat unjointed skin-folds.

Here then was an immense amount of evidence to show that the immediate ancestors of *Apus* must have had upwards of sixty well-developed segments. *Apus* then must stand at the very bottom of the genealogical tree of the modern Crustacea, with their nearly constant number of segments, twenty to twenty-one. Indeed, as above stated, so low did *Apus* appear in the scale of Crustacean organisation that it seemed to form a true connecting link between the Crustacea and the Annelids.

Now this same remarkable character in the segmentation, its fixation at the posterior end of the body in a rudimentary condition, which is unknown in any other living Crustacean besides *Apus*, is visible in many Trilobites. The segments taper away at the end of the body, decreasing progressively in size. So feebly developed, indeed, are the most posterior segments, that I am disposed to consider the pygidium of the Trilobites as a modification largely due to the difficulty of bending such rudimentary segments one upon the other. The earliest known genus, *Olenellus*, has the diminishing segments free to the end, and is, in this respect, even more primitive than *Apus*. The free condition of the segments in *Olenellus* shows that the pygidium,

which sometimes exhibits no traces of segmentation, was in fact a secondary modification.

But this was not all; in addition to the visible posterior tapering away of the segments, Walcott's[1] hypothetical restoration of the under surface, based upon his own and previous discoveries, showed the limbs diminishing in size towards the posterior end of the body, as they do in *Apus*. There was then no escape from the conclusion that the Trilobites were, like *Apus*, derivatives of forms with still richer segmentation than they themselves possessed, and that they, like *Apus*, were shortening their bodies by the fixation of a number of the posterior segments in a rudimentary or larval condition. When, therefore, we added to this the striking resemblances between their head shields, the similarity in form and position of the large labrum, the presence in both of an intestinal tube bent sharply round anteriorly and ventrally so as to open backwards above the labrum, the association of *Apus* with the Trilobites ceased to be a matter of speculation, and it was safe to predict that when actual discovery revealed to us more details as to Trilobite structure, organs would be found homologous with, and probably closely resembling, those of *Apus*. For instance, a pair of minute cirrus-like antennæ could safely be assumed to have existed ventrally on each side of the labrum of the Trilobites, and thus not visible from the dorsal surface.

There was only one objection which might perhaps have been raised against this association of *Apus* with the Trilobites, though hardly by a morphologist. The limbs of *Apus* are phyllopodan, *i.e.*, flat, leaf-like, swimming appendages, while those of the Trilobites, as far as the evidence went, were filamentous, and, according to Walcott's restoration, filamentous even in the rudimentary pygidial segments. This difficulty, by no means insuperable, has, as we shall presently see, been completely disposed of by the recent discoveries.

The first here to be recorded is not one of the

[1] "The Trilobite: New and Old Evidence Relating to its Organisation," *Bull. Mus. Comp. Zool.*, vol. viii., 1880-81.

most recent. I refer to Walcott's discovery of the fossil *Protocaris Marshii*, which shows that *Apus*-like Crustacea were contemporaneous with the very oldest known Trilobites.

Of the new discoveries which have thrown so much light on the details of organisation of the Trilobites, the first was in August, 1893, when Mr. W. D. Matthew announced the discovery of specimens of *Triarthrus Beckii*, showing clear traces of antennæ and of other limbs.[1] The first specimen was discovered by Mr. W. S. Valiant, as long ago as 1884, in the Utica slate, but it was not until 1892 that he found other specimens near Rome, New York, which put the matter beyond question. So fine-grained is the matrix, that not only the limbs, but the setæ on the limbs, have left distinct impressions. This discovery, so long waited for, naturally riveted the attention of those who had studied the subject at all. It is, however, the name of Dr. C. E. Beecher which in the future will be most intimately associated with this advance in our knowledge, owing to his patient unravelling of the finer details of structure and organisation, as these could be gathered from a comparison of many specimens. In a series of papers, he has described, in addition to the antennæ, the limbs of the thorax, the limbs of the pygidium, the limbs of the head, with the metastoma or under lip of *Triarthrus*, and, besides these, some of the posterior limbs of *Trinucleus*, including those of the pygidium.

I propose here to discuss a few of the more important of these new details, and to show how they reveal the true position of the Trilobites in the genealogical tree of the Crustacea.

We owe the discovery of the antennæ and limbs of *Triarthrus* chiefly to the fact that, in that form, these appendages were so well developed as to project all round beyond the edge of the dorsal shield. It is a common phenomenon in the animal kingdom to have one member of a group specialised by the great length of the appendages, all

[1] "On the Antennæ and other Appendages of *Triarthrus Beckii*," *Amer. Journ. Sci.*, ser. 3, vol. xlvi., p. 121.

lengthening alike, the antennæ as well as the legs. The specimens of *Triarthrus*, then, were found with a pair of cirriform antennæ projecting from under the head shield, and a confused fringe of appendages protruding, along each side to the posterior end of the body, from under the dorsal shield. The fact that the antennæ came from *under* the head shield was important, as the antennæ of *Apus* are attached under the head shield on each side of the prostomium or large upper lip, whereas, as a rule, among the higher Crustacea, the antennæ are placed quite anteriorly. And, as a matter of fact, examination of fortunate specimens showed that the antennæ were inserted in *Triarthrus* in practically the same relative position to the prostomium as they are in *Apus*.

If verifications of predictions based upon a theory are proof of the correctness of that theory, here was the proof of the relationship between *Apus* and the Trilobites. That relationship had been based upon other resemblances of organisation, some of the more important of which have been briefly indicated above, and little more than a year after the antennæ of the Trilobites were found exactly where it was predicted they would be found. The importance of this point will be understood when it is remembered that no other known Crustaceans, recent or fossil, except *Apus* and the Trilobites, possess the great square labrum pointing backwards, with the antennæ inserted on each side of it, the whole hidden under a great head shield.

Before passing on to the next point, this ventral position of the antennæ and labrum deserves attention. Occurring in *Apus* with its traces of sixty segments, and in the Trilobites, the oldest fossil Crustaceans, no one can doubt that it is the primitive position of these organs in the crustacean phylum. It follows, therefore, that the annelidan ancestors of the Crustacea, with their originally *anteriorly* placed prostomium, must have bent the first segment sharply round in order to bring that organ into the ventral position found in the primitive Crustacea. There is, in fact, abundance of evidence to show that such a bending did take place. Without going into details which have been

elaborated elsewhere, I may mention a few points, *e.g.*, the sharp anterior bend in the intestinal canal; the curious clumping of the ventral longitudinal muscles, which form the "cartilaginous" endosternites in the angle of the bend; the corresponding stretching out of the longitudinal muscles on the dorsal side; the simple explanation this bending round of head segment affords of the translocation of two pairs of limbs, originally post-oral, into the pre-oral position occupied by the antennæ in the Crustacea; and lastly, the first ontogenetic appearance of the eyes in *Apus* in a more anterior and ventral position than they possess in the adult, and their subsequent wandering upwards on to the dorsal surface. In the discussion of the remaining points which we shall select for mention in the following pages, we shall, therefore, assume that the organisation of these early Crustacea, *Apus* and the Trilobites, can be deduced from that of an Annelid with the first segment bent round ventrally, so that the large prostomium points backwards, while the pair of appendages, specialised into sensory cirri, which were arranged one on each side of it in its original anterior position, remained on each side of it in its new ventral position, and became the first pair of antennæ of the Crustacea. In making this assumption, we are in no sense rushing ahead of our facts; not only does the evidence already brought forward justify us, but the points still to be dealt with confirm it in every instance. We state it thus definitely at this point chiefly in order to facilitate the comprehension of other details.

Having mentioned the large labrum which has long been known, and the antennæ attached at each side of it, the structure next in order, from before backwards, is the under lip, which has recently been described by Dr. Beecher for the first time, as it appears in *Triarthrus*.

The under lip is simply the ventral border of the mouth, but in *Triarthrus* it is bent round as a small ridge projecting backwards. In the original annelidan ancestor, with its anteriorly placed mouth, the dorsal border was formed by the large fleshy labrum or prostomium, while the ventral border, in all probability, showed no specialisation whatever.

As soon, however, as the first segment bent round ventrally, so that the mouth opened backwards, the prostomium would take up the position of the labrum in *Apus* and the Trilobites, and the ventral border would form a small ridge-like under lip, also pointing backwards, such as is actually found in *Triarthrus*. This under lip in *Triarthrus* is thus another slight confirmation of our deduction of the primitive Crustacea from an Annelid with its first segment bent round ventrally.

This under lip of *Triarthrus* is however no longer the simple ridge we must assume it to have been in its most primitive condition. It already shows a tendency to form lateral lobes. These small lobes, so insignificant in themselves, become important when studied comparatively. Their remarkable development in *Apus* reveals to us their function. They supply us with an interesting illustration of the dependence of morphology upon physiological need. In order to make this clear we shall have to make a brief digression to explain the new method of feeding which the bending round of the mouth implies.

The chaetopod Annelids, with their anteriorly placed mouths, largely feed by protruding an evaginable, and generally armed, portion of the pharynx. This is thrust out, seizes the prey, and is then withdrawn back again into the mouth. Any new position adopted by the mouth could only be in adaptation to some new method of feeding. We have, therefore, to see what advantages would be afforded by the bending round of the mouth ventrally backwards. There is an immense amount of nutrient matter, animal and vegetable, which, coating the rocks and weeds, is quite unattainable by the shooting out of a proboscis. Any Chaetopod, therefore, learning to browse in such a way as to be able to take advantage of this hitherto unattainable supply of food, would stand a good chance in the race for life. I have, for instance, long thought that the great phylum of the Mollusca might be deduced, in a manner which I think can be worked out in detail, from Chaetopods which early learnt to feed by scraping the surfaces of things with the ventral edge of the protrusible

pharynx, this, in time, becoming specialised as a radula. And so I believe that the great phylum of the Crustacea arose from Chaetopods also becoming adapted to take advantage of the same food supply, but in a manner very different from, and less efficient than, that adopted by the Mollusca. The ancestor of the crustacean phylum used its appendages as the instruments for obtaining supplies. The food raked from each side by the parapodia into the middle line, was then pushed forward towards the mouth, bent round to receive it. This new function of the parapodia, which were primitively protective on account of their bristles, and locomotory as swimming plates, would soon lead to modifications of structure, which we are not yet in a position to describe in detail. The most primitive phyllopodan limbs with which we are acquainted, *viz.*, the larval limbs of *Apus*, represent the earliest known stage in the transformation of the annelidan parapodium into the crustacean legs. They are flat unjointed skin-folds, carrying on their dorsal edges a pair of processes, one of which has strong claims to be the typical gill of an annelidan parapodium. Their ventral edges are deeply notched so as to form a series of lobes. The proximal lobe of the series is distinct from the rest, and can safely be taken to be the original ventral parapodium.[1] We further find in *Apus* that these innermost lobes (the ventral parapodia), forming a longitudinal series along each side of the middle line, are twisted round in such a way as to show that in addition to assisting to rake food together, they also serve for pushing it forward towards the mouth.

Having thus briefly described the new method of feeding of the ancestor of the crustacean phylum we must return to the ridge-like metastoma of *Triarthrus* and its commencing modifications.

It is not difficult to see that, in its most primitive, ridge-like form, such a metastoma would be a barrier across the ventral surface, hindering the pushing forward of food into

[1] I have figured such a limb, *viz.*, the last limb in *Apus productus*, in *The Apodidæ*, fig. 20, p. 48.

the mouth by the appendages. In order to perfect this method of feeding, this ridge would have to be modified. In *Apus* we find, in fact, that it has almost entirely disappeared in the middle line, while its lateral ends have developed into two fleshy projections looking somewhat like jaws, and often mistaken for such. Thus modified, the metastoma, on the one hand, ceases to be a hindrance against the travelling forward of food along the middle line into the mouth, while, on the other hand, its lateral lobes help to prevent the escape of food on either side when the mouth is reached. Now the metastoma of the Trilobite *Triarthrus* is especially interesting because it still shows the primitive ridge right across the ventral surface, not yet smoothed down in the middle line, while the two ends are only beginning to form their lateral lobes. The discovery of the metastoma of *Triarthrus* is thus another confirmation, this time not only of the relationship between *Apus* and the Trilobites, but also of their common derivation from an Annelid modified in the manner described.

Turning now to the limbs, they offer so many striking confirmations of the same conclusions that they alone are, it seems to me, sufficient to place the relationships here set forth beyond further question. We will briefly note some of the leading points.

(*a*) Our deduction of both *Apus* and the Trilobites from a common annelidan ancestor, from which neither was very far removed, requires that all the appendages except the first pair should have been behind the metastoma. This primitive condition, lost in *Apus* by the metastomial lobes stretching behind the third pair of limbs, is apparently found in *Triarthrus*.

(*b*) The series of appendages behind the metastoma should have been structurally alike, that is, there was no original specialisation of those limbs nearest the mouth into mandibles and maxillæ as distinct from locomotory appendages. This primitive condition is retained by *Triarthrus*.

(*c*) While the first pair of appendages is reduced to a pair of cirri, one on each side of the labrum, the pairs

immediately following are, in the Chaetopods, often reduced in size, progressively increasing till a maximum is reached. This primitive annelidan characteristic is found in *Triarthrus*, as well as in the related *Limulus* and *Eurypterus*; the sixth pair in the last two animals, as well as in *Apus*, being the most powerfully developed.

(*d*) Their hypothetical annelidan ancestor requires that both the Trilobites and *Apus* originally possessed primitive phyllopodan appendages. This has now been fully established by Dr. Beecher's discovery that the rudimentary limbs under the pygidium of two Trilobites, *Triarthrus* and *Trinucleus*, were phyllopodan, like the larval and rudimentary limbs of *Apus*.

(*e*) Such phyllopodan limbs must have extended along the whole length of the body as far as the metastoma. In *Apus* the phyllopodan type persists as far forward as the sixth segment, and can be faintly traced as far as the third; while *Triarthrus* teaches us, as we have seen (*b*), that the homogeneity of the limbs extended originally as far forward as the second pair. We have thus a conclusive proof that the common ancestor of both *Apus* and the Trilobites possessed simple phyllopodan appendages along the whole length of the body from the second segment.

These are some of the new points relating to the appendages as a whole, which arise out of a comparison of Dr. Beecher's discoveries with the known structure of *Apus*. There are many other confirmatory points of interest and importance which, if space allowed, might be adduced, but these are the ones which I have selected to emphasise, because they demonstrate once for all the affinities of these early Crustaceans to one another as common derivatives of an annelidan ancestor, from which neither is far removed.

Having thus briefly traced the Trilobites and their existing ally *Apus* to their common ancestral form, it remains for us to indicate, as far as our knowledge goes, their respective divergent specialisations. However interesting it is to run them back to their common form, it is perhaps even more interesting to trace the lines of advance leading them to their different

fates—the Trilobites to die out with a single survivor in *Limulus*,[1] *Apus* not only to survive to this day, but to give rise to the whole family of modern Crustacea.

Before describing the lines along which the two forms must have diverged, one other very early specialisation of the common racial form has to be mentioned. The bending round of the prostomium with the antennæ, and of the parapodia with their protective bristles, towards the ventral surface, left the dorsal surface quite exposed; some compensatory arrangement for protection was therefore needed. It is not improbable that the dorsal organ, which in the larva is comparatively of great size, and forms a shield-like plate of excretory cells, was protective. In addition to this, however, the skin of the dorsal surface at a very early stage not only thickened, but grew out laterally into folds which roofed the animal over, while the skin of the under surface retained its primitive delicacy and softness, hence its almost universal absence in the fossil Trilobites. The earliest arrangement of these dorsal shields must for the present remain matter of conjecture. The very earliest stage, both ontogenetic and phylogenetic, known to us is that presented by the minute larva of *Olenellus*, the most primitive Trilobite. We there find an enormous skin-fold forming a crescent round the front and sides of the first segment, the three following segments having much smaller lateral folds (pleuræ), which, fusing with this crescent fold of the first segment, together form a roof almost completely protecting the young *Olenellus*.[2] There is some reason to believe that primitively this dorsal roof was confined to the anterior end of the body covering the first five segments. From this common stage the Trilobites, and the Crustacea proper, parted company.

[1] I am inclined to believe that the *Ostracoda* also may be deduced from larval Trilobites in which the head shield folded longitudinally to form the bivalve shell (*cf. The Apodidæ*, p. 256).

[2] From such extremely primitive crustacean or rather pre-crustacean larvæ with their great circular roofs, I would deduce the *Ostracoda*, and the enigmatical palæozoic *Cyclus*.

The Trilobites seem to have perfected the browsing manner of life, spending the whole of their active life in crawling over the sea bottom. The primitive dorsal shield at the anterior end of the body repeated itself on every subsequently developed segment in the form of a pair of sharp blade-like pleuræ. The Trilobites thus browsed securely under a great jointed roof, each joint being provided with a pair of formidable lateral spines. The development of these enormous pleuræ may have early tended to limit the number of segments. In adaptation to this settled creeping manner of life, the phyllopodan limbs became early transformed into filamentous walking legs. The limbs, commencing from the second pair, were used as locomotory (walking) appendages (*cf. Triarthrus, Limulus*). Only under the pygidium, in the rudimentary segments, did a few of the appendages remain phyllopodan. The function of raking together the food, and pushing it forwards to the mouth, must have been entirely carried on by the ventral branches of the parapodia. In *Limulus*, we find these "gnathobases" highly developed, and carrying a cirrus-like appendage, while in *Triarthrus* (*cf.* Dr. Beecher's figures) those nearest the mouth are plate-like jaws, while those on the trunk segments have a remarkable leg-like appearance which can hardly be accidental. I would like to suggest that some special development of these gnathobases might be expected in the Trilobites, that is, *if* the dorsal leg-portions were purely locomotory.

These are a few of the more important points of interest in the specialisation of the Trilobites. The type was plastic enough to give rise to endless small variations, but apparently not plastic enough to re-adapt itself to certain new, and at present unknown, changes in the conditions of life; hence the extinction of the race.

The other great branch of these primitive annelidan Crustacea has had a very different fate. The head shield was not primitively repeated along the body as pleuræ, but grew backwards as a great fold, forming a carapace which protected the back and sides of the trunk. The trunk segments remained simply cylindrical

and persisted for a time in far greater number than in the Trilobites. The limbs further remained phyllopodan as swimming plates. While, however, thus retaining primitive conditions in the trunk, the limbs of the anterior or head segments underwent a very marked specialisation. The first pair remained as simple antennæ, the second pair degenerated and lost their jaw plates, but ultimately developed their sensory functions to become the second antennæ of the typical Crustacean. The third pair lost their locomotory portions and became the powerful mandibles working between the labrum and labium (or labial lobes). The two following pairs also more or less completely lost their locomotory functions, the basal portions persisting as accessory jaws, the maxillæ. This formula for the head limbs, seen in its most primitive condition in *Apus*, became from henceforth the typical mouth formula of the Crustacea.

From such a form as this, all the modern Crustacea can be deduced.[1] The segmentation became more specialised and consequently less rich, the limbs lost their primitive phyllopodan characters, the two pairs of antennæ travelled forwards to the anterior edge of the head; the mouth and mouth-parts also moved forward, causing the almost complete obliteration of the great labrum. The metastomial lobes persist as the "paragnathes," while the great fleshy mandibles of *Apus* became hard chitinous plates. The degenerate locomotory portions of the maxillæ became the palps, while, in some forms, a certain number of trunk limbs, the ventral branches of which, in the primitive form, served to push food forward towards the mouth, move forward and join the maxillæ, as accessory jaws, the maxillipedes. The great carapace has undergone many variations; in some cases it has degenerated, in others it has given rise once more to a system of pleuræ along the trunk by segmental repetition, and forms such as the Isopods have arisen, closely resembling the Trilobites, and perhaps to some extent taking their place in the modern seas.

[1] *Cf.* on this Prof. K. Grobben, *Sitzungsber. d. K. Akad. Wien*, vol. ci., 1892, pt. i.

From this brief account of the rise of the modern Crustacea from *Apus*, it follows that the Trilobites are, in reality, pre-crustacean. They represent a stage more primitive than *Apus*, *i.e.*, nearer to the original annelidan ancestor of the whole phylum.

While the immediate ancestors of *Apus* and of the Crustacea proper retained the power of swimming freely from place to place, the Trilobites became specialised for a creeping manner of life, apparently perfecting along one line the new method of feeding, which, as above described, was the physiological change leading to the subsequent morphological transformation of the Chaetopod into the Crustacean.

We may therefore describe the Trilobites as specialised links in the chain which connects the Crustacea with their annelidan ancestors.

RECENT PAPERS ON THE TRILOBITES.

C. E. BEECHER. On the Thoracic Legs of *Triarthrus*. *Am. Jour. Sci.*, ser. 3, vol. xlvi., 1893.

C. E. BEECHER. On the Mode of Occurrence and the Structure and Development of *Triarthrus Beckii*. *Am. Geol.* vol. xiii., 1894.

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SOME METASOMATIC CHANGES IN LIMESTONES.

THE origin of dolomitic rocks has engaged the attention of geologists and chemists from an early time. Some of the older chemical theories may be dismissed without discussion as having at most a very limited application, and we may confine ourselves chiefly to those views in which the rocks in question are regarded as ordinary calcareous deposits, owing their magnesian character to accidents in the main posterior to their accumulation as strata.

In their present condition these rocks seem to consist in general of dolomite or of a mixture of dolomite and calcite, with which may be associated siliceous, argillaceous, or other impurities in varying amount. Those examples richest in dolomite are styled dolomite-rocks, or simply "dolomites"; those in which the mineral named is subordinate to calcite are correctly termed dolomitic or magnesian limestones, though the latter name has been applied (as in the Permian of the North of England) to strata consisting essentially of dolomite. Cotta fixed the limit at 23 per cent of magnesium carbonate, which corresponds to equal molecular proportions of dolomite and calcite. This percentage should be reckoned, as pointed out by Hopkins (1), on the total carbonates, excluding non-calcareous impurities. We must not exclude the possibility of the simple magnesium carbonate, magnesite, also occurring in some of these rocks, but there seems to be little positive information on this point. However this may be, it seems probable that rocks which on analysis are found to consist of calcium and magnesium carbonates in other than equal molecular proportions, are composed of grains each of which has a definite constitution, *viz.*, that of calcite or that of dolomite (or more doubtfully magnesite). All actual investigations tend to the belief that no true chemical isomorphism exists between the three minerals

named, nor indeed between calcium and magnesium compounds in general. Retgers (2), after a full consideration of this question, came to the conclusion that the carbonates of lime and magnesia examined by him fell under three heads; *viz.*, calcite ($CaCO_3$) with very little or no magnesia, magnesite ($MgCO_3$) with very little or no lime, and the double salt dolomite of perfectly definite constitution ($CaMg_2CO_3$). As a matter of fact, it is not difficult in the less fine-grained magnesian limestones to verify the mixed nature of the rocks by the action of dilute acid, the more soluble calcite being thus separated from the less soluble dolomite.

Between the magnesian and ferrous carbonates, on the other hand, it seems that there is a true chemical isomorphism, just as in the corresponding silicates (both pyroxenes and olivines). Mineral analyses of the rhombohedral carbonates prove the existence of various connecting links between dolomite and pure ankerite ($CaFe_2CO_3$) and probably between magnesite and chalybite ($FeCO_3$). Indeed Bentivoglio (3) goes so far as to maintain that more or less iron is essential to the constitution of dolomite, since in thirty-six analyses of dolomite rhombohedra from various localities, he found ferrous carbonate never absent.

In speculating on the mode of derivation of dolomitic rocks from ordinary limestones various difficulties arise which cannot be said to have been completely surmounted up to the present time, and it is to these especially that we shall turn our inquiries. These difficulties are connected firstly with the source of the large quantities of magnesia which are supposed in most theories of "dolomitisation" to have been introduced from without, and secondly with the chemical processes by which calcium carbonate may conceivably be converted into the double carbonate, dolomite, under natural conditions.

As regards the source of the magnesia, various early writers ascribed to it a volcanic origin. Although it is by no means certain that dolomitisation may not in some cases have been effected in this way, it is manifest that such a hypothesis cannot be of general application. Other geolog-

ists have supposed the magnesia to be introduced in the form of sulphate and chloride from sea-water, and the frequent association of gypsum with dolomite has led to various speculations on a possible reaction between magnesium sulphate and calcium carbonate. The possibility of such a reaction, resulting in the formation of calcium sulphate and calcium-magnesium-carbonate, raises an obvious difficulty, since at ordinary temperatures and pressures the opposite reaction takes place, gypsum and dolomite decomposing one another.

Other writers, again, have inquired whether the magnesia may not have been contained, wholly or partly, in the rock itself from the time of its accumulation; and there are considerations which seem to leave room for difference of opinion as to the extent to which the magnesia has really been supplied from some definitely external source. Many calcareous organisms at the present day contain a noteworthy amount of magnesia, and in some of the calcareous algæ (Lithothamnion) the ratio $MgCO_3 : CaCO_3$ reaches an average of 10 : 100. Several geologists have drawn attention to the large part that has been played by various calcareous algæ in the formation of what are now extensive masses of dolomitic rocks. Von Wöhrmann, for example, has ascribed the thick formations of limestone and dolomite which are so prominent a feature of the Alpine Trias to the agency of algæ of the families Codiaceæ and Siphoneæ (4).

Now dolomite is much less readily soluble than calcite and distinctly less soluble than aragonite; and, if we may assume the magnesia of calcareous organisms to exist in the form of dolomite, it follows that any removal of material under ordinary conditions will leave the residue relatively enriched in dolomite. It has been suggested that the dolomitisation of limestones may sometimes have been effected in this way, rather than by an actual replacement of lime by magnesia introduced in some form from without.

This point has recently been taken up by Högbom in an interesting paper (5). He is dissatisfied with the explanation of dolomitic rocks as ordinary limestones in which magnesian solutions have effected a partial substitution of

magnesia in place of lime, and finds more plausible the suggestion that limestones originally containing some magnesia have become enriched in that constituent by the removal of the lime-carbonate. There seems to be some positive evidence of this process going on to some extent. Thus, while in corals from Bermuda the ratio $MgCO_3 : CaCO_3$ is always under 1 : 100, and in shells still less, the ratio in the reef-rock and lagoon-sediment of the same locality is from 1·7 : 100 to 4 : 100, the higher ratios corresponding to the finer sediments. The author lays stress on the finely divided state of the material, allowing it to remain suspended in water and subjected to the "leaching out" action (Auslaugungsprocess) for a long time. In this connection his examination of the Quaternary muds of Sweden is instructive. These deposits came from the melting of the ice, and doubtless derived their calcareous material from the lower Palæozoic limestones. The latter have $MgCO_3 : CaCO_3$, on the average, in the ratio 1 : 100, while in the muds themselves the ratio ranges from 3 : 100 to 100 : 100 (*i.e.*, to equality). Moreover, on comparing analyses from eight localities, extending from the Gefle district to south of Stockholm, it is found that, as we pass southward, that is away from the source of calcareous matter, while the total carbonates fall steadily from 32·2 to 4·5 per cent., the ratio $MgCO_3 : CaCO_3$ increases steadily from 3·7 : 100 to 36 : 100. Högbom cites also the analyses of deep-sea deposits given in the *Challenger* Report, and shows that as the total carbonates diminish from 87·4 to 4·1 per cent., the ratio in question rises from 0·8 : 100 to 105 : 100.

It must be considered doubtful to what extent the process here discussed is applicable to extensive masses of dolomitic rocks. To convert in this way even a Lithothamnion-rock to a dolomite, as defined by Cotta, would require the removal in solution of four-fifths of the carbonate of lime, or about 74 per cent. by weight of the total material. This can be conceived only in the case of such fine deposits as those specially discussed by Högbom. In dolomitised shore-deposits, in which shells are sometimes bodily replaced by pure dolomite, the greater part of the magnesia

must have been introduced from some external source. It is worthy of note, however, that the matrix of a shelly limestone, which was originally in a finely divided state, seems to be much more readily affected than the enclosed shells.

As regards the possible processes by which calcite can be converted to dolomite by magnesian solutions, various experimental investigations have until quite recently afforded but little assistance. Morlot, in 1847, found that magnesium sulphate and calcium carbonate reacted upon one another at a temperature of 200° C. and a pressure of 15 atmospheres. Marignac, with similar conditions, obtained a reaction between magnesium chloride and calcium carbonate. Hoppe-Seyler, in 1875, obtained a mixture of magnesite and dolomite by the action of magnesium chloride or sulphate on calcium bicarbonate, heated in a sealed tube; the action took place at temperatures between 100° and 160°, but not below 100°. The special conditions necessary for success in such experiments as these make their application to the origin of dolomitic rocks in nature a matter of much doubt. Other possible reactions have been suggested; for instance, that part of a limestone may be converted to magnesite by a solution of magnesium bicarbonate, the excess of lime being removed as calcium bicarbonate, and the magnesite subsequently combining with the remaining limestone to produce dolomite.

More recently Klement (6) has given a discussion of the whole subject of the origin of dolomitic strata, and has made experimental researches which throw an interesting light on some of the difficulties already alluded to in the process of dolomitisation. A brief account of the paper in which his results are embodied will therefore be of some value.

After briefly noticing various former attempts to solve the problem, Klement draws attention to certain ascertained facts which have suggested to him the line taken in his experiments. Typical dolomite, he remarks, is very often found in the form of coral-reefs, and the most complete dolomitisation in the case of atolls. Richthofen has noted in the Trias of the Tirol that the atolls are of dolomite-rock, the barrier-reefs of dolomitic limestone. Dupont, in the

Devonian and Carboniferous of the Ardenne, has specially remarked the dolomitisation of the central parts of the reefs. In the emerged coral-island of Metia or Matea, in the Pacific, Dana has pointed out that the interior, *i.e.*, the part containing the lagoon, is of dolomite-rock with up to 38 per cent. of magnesium carbonate, while the corals of the same reef contain only a trace of magnesia, and he inferred the introduction of magnesia into the rock from sea-water, probably concentrated in a closed lagoon, the process taking place therefore at ordinary atmospheric temperatures. These various facts point to some peculiarity in certain limestones rendering them susceptible to dolomitisation, a peculiarity well exhibited in—though not confined to—rocks of coral origin.

Now the important chemical difference between corals and many other calcareous organisms is that the calcium carbonate in the former is not calcite but aragonite, and Klement was accordingly led to experiment on the action of sea-water on aragonite. The results of his experiments may be summarised as follows :—

Powdered aragonite was acted upon by magnesium sulphate in a saturated solution of sea-salt, the action being found to take place at temperatures above 60° C. The amount of magnesium carbonate formed was found to increase with the temperature, but there appeared to be a certain maximum for each temperature. For 91° the maximum was about 42 per cent. of magnesium carbonate, which is not much less than that in pure dolomite (45·7). In dilute solutions of sea-salt the action was found to be much feebler, and it was also feebler when too much of the solution was used, apart from its degree of concentration. In the absence of sodium chloride the action of the magnesium sulphate, even in concentrated solution, was feeble. Magnesium chloride, substituted for the sulphate, was found to be much less active. The sulphate had very little action on calcite, but acted on corals just as on artificial aragonite.

These experiments go to establish that the magnesium sulphate in sea-water will act upon corals or other aragonite organisms in a warm climate, and in places where the salt in the sea can reach a considerable degree of concentration.

It remains to be inquired whether such action results in the formation of dolomite. This is not a very easy point to settle decisively, but Klement came to the conclusion that the product resulting from his experiments was not dolomite but a carbonate of magnesium alone (neutral but hydrated). He was, therefore, compelled to suppose that there is in nature, not only a formation of magnesium carbonate by a process comparable with his, but further, a combination of this product with the intermixed calcium carbonate to form dolomite.

The crux of Klement's theory is evidently in this latter transformation. There seems to be no direct chemical evidence that such a combination is possible under conditions realised in nature, and the micro-structure of dolomitic rocks does not seem to throw much light upon the question. Our author points to the dolomitisation of crinoidal and other organic bodies as evidence that the assumed combination does take place; but this would seem to prove too much, for crinoids are undeniably calcite organisms. If calcite ossicles of crinoids can be converted into dolomite, which seems to be beyond doubt, we may ask why should not the same transformation affect whole bodies of calcite-rocks, and further, why cannot the process be reproduced in the laboratory?

If the process of dolomitisation of a limestone is something quite independent of the accumulation of the rock, it may presumably be brought about at any distance of time after that accumulation. That it is sometimes long subsequent is proved by its evident connection in various cases with joints, faults, and other geological accidents which have affected the solid limestone strata. Further, the process seems in some cases to have been later than recrystallization in the limestone, whether produced by metamorphism or otherwise. This at least may be inferred from the apparent fact that the dolomitisation seems often to have spared the more coarsely crystalline parts of the rock. The Cambrian limestones in New Jersey, recently described by Nason (7), seem to illustrate this. While the general body of these rocks is of magnesian limestone or true dolomite,

the coarsely crystalline modification near the contact with granitic intrusions is in general non-magnesian. It is perhaps more questionable whether such an idea can be applied to the Durness limestones of the Assynt district in Sutherland, which, according to analyses given by Heddle, are in great part perfectly dolomitised, while the Ledbeg marble, which is their metamorphosed equivalent near the large intrusion of Borolan, is much less magnesian. In these and similar groups of limestones, the facts that certain zones or beds are described as dolomitic, and others alternating with them as non-dolomitic, might seem to suggest that the magnesian nature of the former was closely connected with the accumulation of the deposits. Such an argument, however, is by no means conclusive; we may with equal reason suppose that at certain horizons in the series of deposits there was something in the character of the rocks which predisposed them to the transformation to dolomite. At least the manner in which one and the same bed may sometimes be seen to pass abruptly from a normal limestone to one composed two-thirds of dolomite proves that, in such a case, the magnesian character has been acquired by some secondary change distinctly posterior to deposition.

The well-known concretions of crystalline calcite in some beds of the magnesian limestone of Durham also suggest themselves. Garwood (8) has shown that the ratio $MgCO_3 : CaCO_3$, which in the matrix of these bodies is 66·74 : 100, is in the concretions themselves only 11 : 100. In his view the formation of the concretions is posterior to the dolomitisation, and has been influenced by the amount of magnesia in the several beds, either too high or too low a proportion checking or preventing the development of the structure. The facts, as stated by him, do not, however, seem to be inconsistent with the alternative supposition that the already existing crystalline concretions have resisted the dolomitising process by which the rest of the rock has been transformed.

Leaving these doubtful questions we may pass on to a few remarks on another common metasomatic change, or series of changes, in limestones; that which results in the

replacement of the carbonate of lime by various iron-compounds. It is a familiar fact that very many valuable bodies of iron-ores occur not, like most metalliferous deposits, in the form of lodes and veins, but in more or less distinct strata conformable with the associated sedimentary rocks. Granting that, with suitable conditions, iron-compounds, both oxides and carbonate, may be directly formed as stratified deposits, it is still certain that many important cases demand a different explanation.

The frequent pseudomorphous replacement of calcite by chalybite was pointed out by Bischof thirty years ago, while Blum traced as a further change the conversion of the ferrous carbonate into ferric oxide or hydrate. The chemistry of these processes has been fully discussed by Kimball (9). The geological importance of such transformations has become more clearly recognised, however, since the investigations of Judd on the iron-ores of Northamptonshire and Rutland (1875), Sorby on the Cleveland ironstones (1879), and other geologists on various ferruginous rocks in this and other countries. The derivation of many deposits of chalybite, hæmatite, limonite, and magnetite by metasomatic processes from ordinary limestones is now placed beyond the possibility of doubt.

The number of rocks to which this explanation has been applied is greatly increased by the literature of recent years. As instances we may allude to Lotti's account of the Tertiary iron-ores of Elba (10) and to the descriptions given by American geologists of numerous examples in the United States. Foerste's contribution on the Clinton (Silurian) iron-ores of the Eastern States presents some points of interest (11). These, like many other stratified iron-ores, are often oolitic; but in specimens from numerous localities the author finds the grains to show no true oolitic structure, but to be simply rolled and water-worn fragments of bryozoans. As a rule the iron-ore occurs as a replacement of the substance of the bryozoans themselves, while the cementing calcite and that which occupied the cells may have suffered the same change, and sometimes the cement has been replaced but the organic fragments preserved as

carbonate of lime. Smyth (12) has shown, however, that true oolitic iron-ores do occur in the Clinton group, for example, at the type-locality. Each grain there consists mainly of concentric coats of iron-oxide with a central nucleus of a rounded quartz-granule; but, on removing the iron-oxide with acid, a transparent siliceous skeleton is left, showing a concentric structure, often with ten or more distinct layers. From this intimate association of silica with the iron-oxide, and from other considerations, Smyth concludes that this and some other iron-ores described were formed directly as such, and not by metasomatism from limestones. Some of the difficulties in the way of this view are discussed in Kimball's paper (9), while Smyth has given in another place his reasons for maintaining his view of the probable origin of these Clinton iron-ores (13). It may be observed that the presence of a siliceous skeleton in oolitic grains now composed of ferric oxide or hydrate is a feature of wide-spread occurrence, and is found in ores undoubtedly representing limestone strata. Further, it is met with in oolitic limestones which have not undergone any ferruginous replacement, and is, in this case, possibly only a stage in the complete silicification of the rock.

The oolitic iron-ores of Jurassic age in Lorraine, as described by Bleicher (14), have characters which may probably be taken as typical of many other occurrences. As regards their origin the author expresses no opinion, but contents himself with quoting Braconnier's supposition that the material (now hæmatite) was deposited as chalybite brought in solution by water containing excess of carbonic acid. Each grain seems to consist of concentric coats of ferric oxide, more or less argillaceous, investing a central grain. The latter is often organic, consisting of a fragment of a bryozoan, foraminifer, or shell, more or less mineralised and impregnated with iron. The grains, when treated with acid to remove the hæmatite, and heated, became white or grey and transparent. They then consisted merely of a nucleus of siliceous material surrounded by numerous delicate concentric shells, some thin and clear, others cloudy

and enclosing minute sand-grains. These shells were found to be of silica, soluble in caustic soda. With a high magnification they showed very minute rods, which, however, might probably be foreign bodies introduced during the preparation of the specimens.

It is evident that since bedded iron-ore deposits may admittedly be formed either directly as such or indirectly by the transformation of limestones, the origin of any particular deposit must be a question to be decided by such evidence as is available for the case in question. Some of the criteria which may be applied in discussing the question are well brought out in the papers of Kimball and Smyth on the Clinton ores. For instance, Smyth, in maintaining the direct formation of these rocks, points to the rarity or absence in the wide area discussed by him of chalybite, which, on the metasomatic theory, must be formed as a stage in the transformation of limestone to hæmatite. He considers that, if this latter theory were the true one, there should be, at least in some places, ferrous carbonate remaining to mark the intermediate stage of the transition. The normal ore is a red hæmatite, usually of earthy aspect, but sometimes quite compact and with an almost metallic lustre. One type consists largely of fragments of bryozoa, corals, crinoids, brachiopods, etc., coated by, and more or less converted to, hæmatite. The oolitic type is less abundant.

It will be observed that Smyth himself admits some extent of metasomatic change, at least as regards the organic fragments in the Clinton ores. The replacement especially of bryozoan and coral remains is a common feature in many limestones, *e.g.*, in the Carboniferous of the Bristol district. Again, it is not uncommon to find oolitic grains more readily affected than their matrix, and in many of the English oolitic iron-ores we may observe that the grains have been converted into hæmatite or limonite, while the matrix is only in the condition of chalybite.

The metasomatic processes in calcareous rocks which result in what may be termed silicification open out a number of interesting questions which cannot conveniently be treated in the present contribution, and the apparent re-

placement of limestones in some areas by ores of manganese, zinc, etc., must also be left to a future occasion.

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THE DECOMPOSITION PRODUCTS OF PROTEIDS.

AN intimate knowledge of the constitution of Proteids would lead to such important and far-reaching physiological results, that many observers have been induced to undertake lengthy investigations with a view, if possible, to solve or to throw some light upon this difficult problem.

A question such as this, purely chemical as it is in its bearings, must be attacked from a chemical standpoint by a study of the cleavage products of proteids, and just as our knowledge of simpler organic bodies is only placed upon a sure basis by a successful synthesis, so, too, the constitution of the proteid molecule can only be finally and satisfactorily determined by the synthesis of albuminous substances. Before this can be effected it is, however, necessary to ascertain what substances are formed, and the quantities in which they are produced by the disintegration of various proteids.

It is chiefly to a study of this nature that experimenters have as yet applied themselves. At the outset we may classify the experiments as falling into two main groups, the first designed with a view to determine the series of changes a proteid undergoes during its transit through the body, the second with the object of investigating the chemical substances produced as cleavage products by artificial means *in vitro*. In the first category the progress made has been but slight, because of the great and obvious difficulties encountered at every step. We know the end products of the full series of changes, *viz.*, carbonic anhydride and water from the carbon and hydrogen respectively, and from the nitrogen urea, uric acid, ammonia, and other more complex nitrogenous products, occurring either in small quantities or only under special conditions. These result from changes within the tissues and cells of the body, but with regard to the intermediate substances occurring between these at the one extreme, and the com-

plex proteids of the blood and tissues at the other, we are still in the region of conjecture.

There is, however, another series occurring in the alimentary tract which has yielded more plentiful results. As early products we have been taught to distinguish proteids, albuminates and peptones, and many simpler nitrogenous bodies like leucine and tyrosine.

It is mainly, however, from changes brought about by the treatment of proteids with varying reagents that we have gained our greatest knowledge on this subject. In this respect the aim must be to isolate all the products produced during the various decompositions, and so obtain a complete series which will show us, step by step, the succession of changes the proteid has undergone. It is my intention to indicate in this paper how some of these steps have been attained, and to point out the arguments which may be based upon the knowledge thus gained.

Most of the researches as yet carried out have investigated the early or the final products. The changes produced by the first action of the different reagents have resulted in the formation of albuminates, proteoses, and peptones. Into these changes it is not necessary to enter in detail. In considering the final products formed, it will be convenient to arrange the subject under the headings of the different methods employed.

1. *Alkalies.* Mulder[1] treated albumin with caustic potash, and obtained a body to which he gave the name "protein," and which he considered as the base of all albuminous material. He also described various compounds of this substance such as sulpho-proteic acid, protein, etc., but most of his assertions have been disproved by Liebig,[2] who showed that "protein" is a body which we now know as alkali-albuminate. Pavy states that by the action of caustic potash upon albumin he has been able to split off a body of an amylose nature, which on further treatment yields a reducing sugar.

[1] *Journ. f. praktische Chemie*, vol. 16, p. 129, and vol. 17, p. 312; also Liebig's *Annalen der Chemie*, vol. 31, p. 129.

[2] Liebig's *Annalen der Chemie*, vol. 57, p. 132.

O. Nasse introduced the method of boiling proteids with a strong solution of barium hydrate, a method which, elaborated by Schützenberger, has yielded many valuable results. Nasse[1] discovered that under these circumstances some of the nitrogen was disengaged as ammonia, but that this accounted (in all cases with the exception of gluten) for but a small percentage of the total nitrogen. As a result of his experiments he came to the conclusion that the nitrogen of a proteid is held under different conditions; some which is readily eliminated by the alkaline earth is in the form of an amide, some is similar to the nitrogen of a creatine group, but the major part, that which is unaffected by this treatment, is in the form of an amido-acid.

Schützenberger[2] in a series of researches extending over many years has considerably amplified this method. By varying the conditions of temperature and pressure, of amount of barium hydrate employed, and the length of the time of treatment, he has obtained differing results. In his earlier researches he employed coagulated albumin from egg-white which was thoroughly washed with water, alcohol and ether. Weighed amounts of this were treated with quantities of crystalline barium hydrate varying from two to six times its weight, and with water in a closed iron vessel, the whole being heated to temperatures ranging from 100° C. to 250° C., and during times which varied in different experiments from eight to 120 hours. A very large number of such experiments were carried out, and he was able to confirm Nasse's results with regard to the quantity of ammonia given off. Extending his observations he found (i.) that nitrogen to the extent of about 1 per cent. of the total weight of albumin was given off as ammonia by boiling for about half an hour at ordinary pressures; (ii.) that another 1 per cent. is slowly given off by continued boiling for 120 hours, and that this second result can be

[1] *Chem. Centralblatt*, p. 137, 1873. And Pflüger's *Archives*, vol. vi., p. 589; vol. vii., p. 139; and vol. viii., p. 381, 1872.

[2] *Ann. de Chim. et de Physique*, 5th series, vol. 16, p. 289 (1879). *Comptes Rendus*, vol. 101, p. 1267; vol. 102, p. 289; vol. 106, p. 1407; and vol. 112, p. 189. *Bulletin de la Soc. Chim.* (1875), and vol. 24.

more easily obtained by treating with three parts of barium hydrate at 120° C. for six to eight hours; (iii.) a third 1 per cent. is given off by treating with two parts barium hydrate for twelve hours at 150°, and (iv.) a fourth 1 per cent. is obtained by heating with excess of barium hydrate for several hours at 260°.

He next found that accompanying these four stages of the decomposition there were different cleavage products obtained. First, there were some insoluble barium salts, *viz.*, carbonate, oxalate, phosphate and sulphate. On calculating the quantities of the carbonate and oxalate formed, he arrived at the interesting result, that they were present in just those proportions to confirm the hypothesis, that, with the ammonia set free, they had existed in the proteid molecule as a urea, and oxamide radicle. The barium carbonate and oxalate, moreover, were formed at different stages of the elimination of ammonia, and by combining the results obtained, he was led to the view, that the first amount of ammonia came from one of the amide radicles of the oxamide, the second corresponded especially to the urea, and the third to the remaining nitrogen of the oxamide (present then as oxamic acid).

After precipitating the barium with carbonic anhydride and sulphuric acid, he obtained, by distillation in a partial vacuum, a small quantity of acetic acid mixed with traces of formic acid, and an essential volatile oil which he identified as pyrrol contaminated with smaller quantities of methyl- and ethyl-pyrrol.

The remaining bodies did not volatilise nor sublime at low temperatures, and these he termed the "résidu fixe". By contrasting the elementary analysis of this with that of the original albumin, and taking into account the quantities of the above-mentioned substances, he was enabled to prove that the essential action of the barium hydrate was one of hydrolysis.

For the qualitative analysis of the "résidu fixe" he relied entirely upon processes of isolation by repeated crystallisations from water, alcohol, or ether, after he had spent much time in endeavours to effect the same object by precipita-

tion with basic and neutral lead acetate, or mercuric nitrate, but without success.

Employing this method he showed that the whole of the "résidu fixe" consisted of amido-acids. These are of two classes :—

(A) In the first, which comprises over 80 per cent. of the total weight, the ratio of N : O is equal to 1 : 2, and consists of bodies of the following classes :—

(i.) Amido-acids of the series $C_nH_{2n+1}NO_2$. These he termed leucines. Of these were found Alanine (C = 3) in small quantities only, Propalanine or amido-butyric acid (C = 4), Butalanine or amido-valeric acid (C = 5), both in considerable quantities, and Leucine or amido-caproic acid (C = 6) in very large quantities. It is to be noted that glycocine (amido-acetic acid) was not found.

(ii.) Amido-acids of the series $C_nH_{2n-1}NO_2$. These belong to the acrylic acid series, and Schützenberger called them Leucëines. Here, too, the term occurring most abundantly is (C = 6) "caproic Leucëine," but bodies corresponding to C = 5 or 4 were also found.

(iii.) Amido-acids of the form $C_nH_{2n}N_2O_4$ or some multiple of this. To these bodies he gave the name of glucoproteins. The most abundant of these are those in which C = 9 and C = 7 or some multiple, but others where C = 11, 10, 8 have also been isolated.

(B) In the second the ratio of N : O is 1 : 3 or 1 : 4 or 2 : 5. It only contains about 16 per cent. of the total weight. In this class are found :—

(i.) Tyrosine $C_9H_{11}NO_3$ or oxyphenyl-amidopropionic acid. The amount of this is about 3·5 per cent.

(ii.) Nearly the same quantity of a body Tyroleucine $C_7H_{11}NO_2$.

(iii.) Very small quantities of glutaminic acid $C_5H_9NO_4$. This is an amido-acid (optically inactive) of one of the pyrotartaric acids (glutaric).

Of these substances Schützenberger found varying

quantities, according to the degree to which the hydration had been carried out. If this had been thorough the final bodies, *viz.*, the leucines and leucëines, were found, but if it had only been partial large quantities of glucoproteins were obtained. He also found evidence of combinations of these with leucines or leucëines to form still more complex bodies. He therefore concludes that the albumin molecule under the action of barium hydrate loses ammonia, carbonic anhydride, acetic and oxalic acid, and becoming hydrated forms first glucoproteins, mainly $C_9H_{18}N_2O_4$ or more probably a multiple of this, and on further action these become converted into bodies of the leucine and leucëine type.

He has further extended his observations to other proteids and shown that they give almost identical results.

Under the same treatment gelatine, icthyocoll and ossein were found to yield the same bodies, with the addition of amido-acetic acid (glycocine) in very large quantities (20-25 per cent.).

2. *Acids.* Prolonged heating of proteids with dilute acids was found by Neumeister[1] to result in hydration and the production of proteoses and peptones. I have observed the production of the same bodies by the action of strong acids acting for several days at the temperature of the room. The prolonged boiling with a strong solution of sulphuric or hydrochloric acids yielded ammonium salts, leucine, tyrosine, aspartic and glutaminic acid. This method had the great disadvantage that many strongly coloured bodies appeared, and to avoid this Hlasiwetz and Habermann[2] introduced the important modification of heating with strong hydrochloric acid and stannous chloride, by which pale yellow solutions were obtained which showed no trace of charring. At the end of the process most of the stannous chloride was found converted into stannic chloride, showing that a certain amount of reduction had occurred. Casein was the proteid at first employed by them in their researches, and from the liquid so obtained they were able to isolate :—

[1] *Zeitschr. für Biologie*, vol. 23, p. 381.

[2] Liebig's *Annalen der Chemie*, vol. 169, p. 150, 1873.

(i.) Ammonia.

(ii.) An amido-acid of the acetic series, *viz.*, leucine.

(iii.) Two amido-acids of the acrylic series, *viz.*, asparaginic acid $C_4H_7NO_4$ (amido-succinic acid), and glutaminic acid $C_5H_9NO_4$ (amido-pyrotartaric acid), both of which were found in considerable quantities. On one occasion the glutaminic acid amounted to as much as 29 per cent. of the weight of dry casein used.

(iv.) Tyrosine or oxyphenyl-alanine.

It is thus seen that, as with the action of barium hydrate, the result of this decomposition is to lead mainly to the formation of amido-acids.

Ritthausen[1] was the first to separate glutaminic and asparaginic acids from proteids. He obtained glutaminic acid from legumin and conglutin, and Kreusler examined animal proteids treated in a similar manner, but failed to isolate it. He consequently suggested the view that it was a typical product of the decomposition of vegetable-, but absent from animal-proteids. Hlasiwetz and Habermann,[2] however, obtained it in considerable quantities from casein, albumin, and several other proteids. It is interesting to note that the glutaminic acid here isolated is lævorotatory, whilst that obtained by Schützenberger was only found in small quantities and inactive. By boiling the active form with barium hydrate, however, it becomes converted into the inactive form, and further, this inactive form, or that obtained by Schützenberger's process, if allowed to ferment under the action of *Penicillium glaucum*, becomes transformed into the optically active modification.[3]

Employing the same method, Horbaczewski[4] obtained from gelatin, leucine, glutaminic acid and glycocine, but

[1] *Journ. f. praktische Chemie*, 107, p. 218, and 99, p. 454.

[2] *Anz. der K. Akademic zu Wien*, ix., p. 114, 1872.

[3] *Berichte der deutschen Chemischen Gesellschaft*, 17, 388. Changes in the optical varieties of leucine have been produced by exactly similar methods.

[4] *Sitz. der K. Akad. zu Wien*, bd. 80, Abthlg. 2, s. 101, 1880.

obtained no asparaginic acid nor tyrosine. Hofmeister[1] has, however, obtained small quantities of asparaginic acid. From elastin, Horbaczewski[2] has obtained by the same method ammonia, leucine, tyrosine, glycocine and amido-valerianic acid $C_5H_{11}NO_2$ (butalanine), but no glutaminic nor asparaginic acids. Siegfried, working on a substance he obtains from reticular tissue, and which he calls "reticulin," obtained ammonia, sulphuretted hydrogen and amido-valerianic acid, but no tyrosine nor glutaminic acid.

By the employment of this same method, Drechsel has discovered two new bodies, lysine and lysatinine, which are of especial interest. He was induced to take up this question by a consideration of the quantities of the different nitrogenous bodies which had been obtained. Assuming that only one half of these bodies had been isolated by the methods hitherto employed, there still remained about 30 per cent. of the original nitrogen to be accounted for. Now contrasting Hlasiwetz and Habermann's results with those obtained by Schützenberger, Drechsel[3] points out that carbonic anhydride was produced by the latter method but never by the former, and he therefore argues that there must be some other body which should be capable of isolation, and which on treatment with barium hydrate will yield carbonic anhydride.

We have already seen that Schützenberger[4] considers this would be of the nature of a ureïde. Considering it probable, therefore, that this substance might be of a basic nature, he determined to first ascertain whether he could isolate it as a precipitate by phosphotungstic acid, being led to employ this reagent on account of its known value as a precipitant of bodies of an alkaloidal or basic nature. In this expectation he was completely successful and obtained a very bulky precipitate from which he was able to isolate two bases, which he named lysine and lysatinine.

Lysine has a formula $C_6H_{14}N_2O_2$ which is homologous

1 *Zeitschr. f. physiol. Chemie von Hoppe-Seyler*, vol. ii., p. 299.

2 *Sitz. der K. Akad. zu Wien*, bd. 92, Abthlg. 2, s. 657, 1886.

3 *Der Abban der Eiweiss-stoffe du Bois' Archiv*, p. 248, 1891.

4 *Loc. cit.*

with Jaffé's[1] ornithin $C_5H_{12}N_2O_2$, which is probably diamido-valerianic acid. Drechsel considers therefore that probably lysine is a body of this class (diamido-caproic acid, for instance).

If this base be heated with strong hydrochloric acid up to a temperature of 150° C., no change occurs; but on heating it with barium hydrate to 120° or 130° it is decomposed with the production of barium carbonate. It can, therefore, account for some of the carbonic anhydride observed in Schützenberger's experiments.

The chief interest centres, however, round the second base, lysatine or lysatinine, which has a formula either $C_6H_{13}N_3O_2$ or $C_6H_{11}N_3O$, and is a homologue of either creatine or creatinine $C_4H_9N_3O_2$ or $C_4H_7N_3O$, according as the first or second formula is taken. It is obtained pure as a double salt of its nitrate in combination with silver nitrate, and this double salt when boiled with barium hydrate for twenty-five minutes yields urea. This is a decomposition exactly analogous to that of creatinine under the same circumstances. From ten grammes of the double salt he obtained about one gramme of urea, a considerable quantity when one considers that urea itself under these circumstances is quickly broken up into ammonia and carbonic anhydride. The great importance of these facts rests in that this is the first time urea has been obtained as a direct decomposition product of a proteid.

Béchamp,[2] viewing metabolism as essentially a process of gradual oxidation, considered he ought to get urea by acting upon proteids in such a manner. He therefore treated dried egg-white with potassium permanganate and stated that he had been successful in obtaining small quantities of urea. This result was contradicted by Stædeler,[3] Lœw,[4] and Tappeiner,[5] though confirmed by Ritter.[6] The matter was, however, finally settled by the experiments of

[1] *Ber. der deut. chem. Gesellschaft*, vol. x., p. 1925.
[2] Liebig's *Ann. Chem.*, vol. 100, p. 247, 1856.
[3] *Journ. f. Prak. Chemie*, vol. 72, p. 251, 1857.
[4] *Ibid.* (2), vol. iii., p. 289, 1871.
[5] *Ibid.* (2), vol. iv., p. 408, 1871.
[6] *Comptes Rendus*, 73, 1219.

Lossen,[1] who found that the substance Béchamp and others had obtained was not urea, but guanidine, which is readily mistaken for it. This guanidine might have originated from small quantities of xanthine present as an impurity in the egg-white employed, for xanthine on oxidation with hydrochloric acid and potassium chlorate produces guanidine. On the other hand Drechsel[2] points out that creatine is a methyl-guanidine acetic acid and that when treated with mercuric oxide it yields methyl guanidine; and assuming lysatinine to be a homologue of creatinine, the guanidine obtained by Lossen may have come from that, and Drechsel hopes to be able, by treatment with mercuric oxide, to obtain it from lysatinine. Drechsel is careful to point out that the urea obtained by his method is not a result of oxidation, but of hydration processes. The stannous chloride effectively prevents oxidation, and, moreover, the action of the barium hydrate on lysatinine is again a hydration.

The remaining products obtained from the lysatinine have not yet been isolated.

Drechsel, therefore, considers that one possible source of urea as a result of proteid metabolism may be lysatinine. But supposing that all the proteid assimilated yielded the proportion of lysatinine, and then of urea, that he found in his experiment *in vitro*, even then only about one ninth of the total urea excreted will be accounted for.

There is, however, another point which has not been noticed, but which I think gives us a reason why we should not expect to find urea among the decomposition products of proteids. The whole of the knowledge we at present possess tends to show that urea is not formed in those positions in the body where proteid disintegration is occurring, *viz.*, the tissues, but that it is formed in one position only, the liver, and there too mainly, if not exclusively, from the very simplest nitrogenous bodies, ammonium salts. Looked at from this point of view the series of

[1] *Ann. Chem. Pharm.*, vol. 201, p. 369, 1880.
[2] *Loc. cit.*

changes a proteid seems to undergo within the tissue cells result in the formation of ammonia, not of urea. This ammonia passing into the lymph and blood combines with carbonic acid to form either carbamic acid or carbonate of ammonium. The production of carbamic acid especially occurs if the fluid is alkaline. Drechsel[1] has found it in the blood-serum, and Drechsel and Abel[2] have found it in horses' urine.

Schröder's experiments have shown that ammonium carbonate added to blood circulating artificially through an isolated liver, is directly converted into urea. This is confirmed, and the view that this is the normal method within the body is upheld by the experiments in which the urea and ammonium salts have been quantitatively determined in the urine. When a strong mineral acid is administered so as to render the blood plasma less alkaline, more and more of the eliminated nitrogen appears as the ammonium salt of a mineral acid (sulphate, chloride, etc.), and less as urea. And we know, further, that ammonia presented to the liver cells, in combination with strong mineral acids, is not altered by them.

The changes occurring, then, to proteids within the body are first hydrolisation, and then the carbon is split off, atom by atom, and burnt to form carbonic anhydride. That this gradual disintegration is possible is shown by a previous research of Drechsel's[3] relating to the action of strong alternating currents upon caproic acid. The alternating current acts upon the solution so as to produce rapidly changing series of oxidations and reductions. By an examination of the results Drechsel found that from the caproic $CH_3.(CH_2)_4CO.OH$, oxy-caproic $CH_2.OH.(CH_2)_4CO.OH$ acid arose by oxidation, from this adipic acid $CO.OH(CH_2)_4CO.OH$ arises by oxidation, and on oxidation of this oxy-valerianic acid $CH_2OH(CH_2)_3CO.OH$ and carbonic anhydride are produced, and so on till all the carbon atoms are burnt off as carbonic anhydride.

Again, from oxy-valerianic acid $CH_2OH.(CH_2)_3CO.OH$

[1] *Sitz. der Königl. Sächs Gesell.* Sitzung vom., 21 April, 1875.

[2] *Du Bois' Archiv*, p. 236, 1891.

[3] *Journ. Prak. Chemie* (2), vol. 34, p. 135.

by reduction valerianic acid $CH_3(CH_2)_3COOH$ is produced. All these bodies have been identified by Drechsel, and he moreover shows that the process can be carried out to the end, when all the carbon appears as carbonic anhydride, the hydrogen as water.

On similar treatment of an amido-acid a similar series is observed, the nitrogen being ultimately found as ammonium carbonate.

Further the same process will convert ammonium carbonate into urea.[1]

This series of changes will thus help us to understand the formation of several terms in the homologous series isolated by Schützenberger, and the contrast of but few of the terms of the series isolated after treating proteids with strong acids.

Drechsel obtained lysine and lysatinine from casein, but working under his direction several of his pupils have discovered them among the cleavage products of other proteids. Fischer[2] obtained them from gelatin. Siegfried obtained them from conglutin[3] and from reticulin.[4] Siegfried working with conglutin also obtained a body of formula $(C_8H_{16}N_2O_4)_n$ of sweet taste, and answering to one of the bodies described by Schützenberger as a glucoprotein.

3. *The action of oxidising agents.* Treatment with nitric acid has yielded oxalic, fumaric, and saccharic acids. By its early action a yellow insoluble substance, xanthoproteic acid, is first produced, but this gradually dissolves, and finally there is produced para-oxybenzoic and oxybenzoic acids. These latter are probably due to the oxidation of tyrosine.[5]

Oxidation with manganese dioxide and sulphuric acid, or with potassium bichromate and sulphuric acid, has yielded—

[1] Drechsel, *Journ. f. Prak. Chemie* (2), vol. 22, p. 476.

[2] *Inaugural Dissert.*, Leipzig, 1890; and *Du Bois' Archiv*, p. 265, 1891.

[3] *Ber. d. deut. chem. Gesell.*, vol. xxiv., p. 418.

[4] *Ueber die chemischen Eigenschaften des reticulirten Gewebes* (*Habilitationschrift*), Leipzig, Dec., 1892.

[5] See Baumann, *Ber. d. deutsch. Chem. Gesellschaft*, 12, 1453.

(i.) Formic acid, acetic acid and the homologues of these fatty acids up to caprylic acid $C_8H_{16}O_2$.

(ii.) The aldehydes of these acids.

(iii.) The nitriles of acetic, proprionic, valeric, and butyric acids and hydrocyanic acid.

(iv.) Benzoic acid and benzoic aldehyde.

When oxidised with chlorine water they yield among other bodies, fumaric and oxalic acids, and chlorazol.

Oxidised by bromine water at high temperatures in a sealed tube, Hlasiwetz and Habermann[1] isolated from among the products formed carbonic anhydride, oxalic acid, ammonia, bromanil ($C_6Br_4O_2$), bromoform ($CHBr_3$), monobrombenzoic acid, mono- and di-bromacetic acids, tribromamidobenzoic acid ($C_6HBr_3(NH)COOH$), asparaginic and malaminic acids, leucine, and leucimide ($C_6H_{11}NO$). No tyrosine was obtained.

Bleunard,[2] working with the results of the action of bromine, has been able to confirm the production of Schützenberger's glucoproteins.

4. *By dry distillation* of proteids a complex oil, "Dippel's oil," is produced which contains among other substances :—

(i.) Hydrocarbons of the fatty series.

(ii.) Ammonium salts of the fatty acids, *e.g.*, butyric, valeric, caproic.

(iii.) Nitriles of this series from propyl nitrile to stearyl nitrile.

(iv.) Ketones of this series.

(v.) Carbonic anhydride.

(vi.) Ammonia and amines of fatty acid radicles, *e.g.*, methylamine, propylamine, butylamine.

(vii.) Hydrocarbons of the benzene series.

(viii.) Amines of this series. Aniline and its homologues.

(ix.) Phenol and its homologues.

(x.) Homologues of the pyridine group of bases:

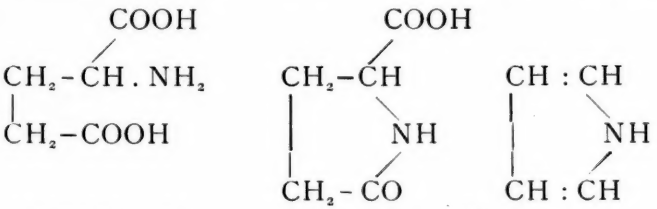
[1] Liebig's *Ann. d. Chem.*, 159, p. 304, 1871.

[2] *Comptes Rendus*, vol. 90, p. 612, and vol. 92, p. 458.

Pyridine ($C_5H_5N_5$), Picoline (C_6H_7N), Lutidine (C_7H_9N), and Collidine ($C_8H_{11}N$).

(xi.) Pyrrol. Of feebly basic properties.

Perhaps the most interesting of these are the pyridine group and pyrrol; the former because they have been shown to take a part in the molecular formation of many vegetable alkaloids, *e.g.*, piperidine, cinchonine, etc. As an example which will show us the manner in which the bodies described as decomposition products of proteids by acids and alkalies could become converted by distillation into bodies of closed rings, such as the last two mentioned classes, I will take the conversion by heat of glutaminic acid into pyrrol. At 190° dry glutaminic



glutaminic acid. pyroglutaminic acid. pyrrol.

acid is converted into pyroglutaminic acid, and at a higher temperature this in its turn becomes converted into pyrrol.[1]

5. *Decompositions occurring by ferment action.* First among these we have the earlier stages of hydration of the proteid, *viz.*, the proteoses and peptones; but in addition to these it has been shown that the action of trypsin may extend further. The other substances known to be produced may be classified into :—

(i.) Bases. Lysine and lysatinine have been shown by Hedin,[2] working under Drechsel, to occur among these.

Hirschler[3] obtained evidence of the production of ammonia, a result confirmed by Stadelmann[4] under conditions precluding the possibility of putrefaction.

[1] *Ber. der deutsch. chem. Gesell.*, xv., 1222.
[2] *Du Bois' Archiv*, p. 273, 1891.
[3] *Zeitschr. f. physiol. Chem.*, vol. x., p. 302, 1880.
[4] *Zeit. f. Biologie*, vol. xxiv., p. 261, 1888.

(ii.) Amido-acids. Of these leucine, amido-valerianic (butalanine), amido-succinic (aspartic), and amido-pyrotartaric (glutaminic) acids have been isolated.

(iii.) Aromatic bodies, tyrosine or oxyphenyl-alanine. It is thus seen how closely these results correspond with those obtained by artificial decomposition.

Within the intestine many changes occur which have been found due to decompositions under the influence of bacterial action. The same products, as above enumerated, are first formed, but by further and different changes other bodies rapidly arise. Of these we may mention—indol, skatol, skatol-carbonic acid, oxyphenyl propionic acid, oxyphenyl-acetic acid, phenyl propionic and phenyl-acetic acids, para-kresol and phenol, and as simpler bodies carbonic anhydride, water, ammonia, hydrogen, and sulphuretted hydrogen, in addition to amido-acids of the fatty series and the fatty acids themselves.

In this case certainly the most interesting point to note is the production of so many derivatives containing the benzene nucleus. The indol group has never been obtained from a proteid molecule by any other method.

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